Original Article

Drug susceptibility profiling of pulmonary *Mycobacterium kansasii* and its correlation with treatment outcome

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Abstract:

OBJECTIVES: With the introduction of newer molecular diagnostic tools to identify *Mycobacterium tuberculosis*, an increasing number of nontuberculous mycobacterium (NTM) is being identified. However, the drug resistance pattern of the NTM species identified is less explored. The objective of this study is to study the drug resistance patterns of *Mycobacterium kansasii* species isolated in a tuberculosis-endemic setting at South India.

METHODS: A wide profile of NTM species were reported earlier from a prospective cohort of adults during 2017–2020. Out of this profile, a total of 22 *M. kansasii* species were subjected to drug susceptibility testing by two different methods: proportion sensitivity testing method and Sensititre testing method.

RESULTS: Out of the 18 strains of *M. kansasii* subjected to Sensititre method of testing, the resistance pattern was demonstrated to be high for doxycycline (13) followed by rifampicin and trimethoprim/sulfamethoxazole (7). Out of the 22 strains subjected to proportion sensitivity testing method, 20 and 10 were resistant to isoniazid and ethambutol, respectively.

CONCLUSION: There was a poor correlation between the treatment outcome and the resistance pattern of the antibiotics tested. With increasing numbers of NTM being reported, early and correct identification of NTM species is essential for the prompt initiation of appropriate treatment to achieve better outcome.

Keywords:

Drug regimen, drug susceptibility testing, *Mycobacterium kansasii*, misdiagnosis, nontuberculous mycobacterium, tuberculosis

Nontuberculous mycobacteria (NTMs) that were earlier considered as the nonpathogenic, environmental mycobacteria are gaining importance in recent years. *Mycobacterium kansasii*, known to cause both pulmonary and extrapulmonary infection, is one of the six most frequently isolated NTMs. This species is considered to be the most pathogenic NTM, with the majority of culture-positive cases presenting with clinical disease, along with the colonization.[1-4] Among the seven subtypes (I–VII) identified so far, subtype I is associated with human infections, and subtype II is associated with HIV-infected patients indicating its role as an opportunistic pathogen.[5] The other five subtypes are, in general, documented as environmental isolates rather than pathogenic species.[6] The perceptible similarities between clinical presentations

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of pulmonary *Mycobacterium tuberculosis* (MTB) and *M. kansasii* have made the physicians to rely on microbiological confirmation to distinguish between these infections.[7-9] To diagnose pulmonary NTM, a minimal radiological evaluation with a chest X-ray (or computed tomography), combined with positive cultures and clinical exclusion of other diagnoses, is recommended by the American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) guidelines.[10] Identification of pulmonary *M. kansasii* with this combined diagnostic algorithm of clinical and microbiological investigation along with its drug susceptibility testing (DST) could facilitate timely and proper management of the symptomatic patient.

The different types of DST methods currently used for *M. kansasii* include broth microdilution, agar proportion, and E-test methods. These methods vary by different factors such as inoculums and medium used, incubation conditions, and interpretation of the results, thereby influencing the outcomes.[11] DST of *M. kansasii* is a less explored topic, and as of other mycobacteria, the Clinical and Laboratory Standards Institute (CLSI) recommends the microdilution method in Mueller–Hinton medium with drugs rifampicin (RIF) and clarithromycin (CLA) as for now.[12] The other drugs isoniazid (INH), ethambutol (EMB), streptomycin (STR), amikacin (AMK), co-trimoxazole (SXT), rifabutin (RFB), moxifloxacin (MXF), linezolid (LZD), and ciprofloxacin (CIP) are recommended for testing only in the exceptional cases of RIF resistance.[13,14] We recently reported a profile of NTMs that were isolated from a cohort of adults with symptomatic pulmonary disease.[15] In this cohort, *M. kansasii* was isolated from sputum specimens in 46.8% (22/47) of patients with presumptive pulmonary tuberculosis (TB). In this study, we aimed to determine the drug susceptibility pattern of the *M. kansasii* strains using SLOWMYCO Sensititre plates (TREK DIAGNOSTIC Systems Ltd., UK) and correlate it with the clinical outcome.

Methods

Study population

A cohort of adults with respiratory complaints similar to pulmonary TB and with high suspicion of pulmonary NTM disease was referred to our institute during 2017–2020. They were smear positive for acid-fast bacilli (AFB) but were negative for MTB by culture and hence referred to us for further evaluation. Prior approval by the institutional ethics committee was obtained for the study.

Detailed clinical and demographic details including occupation were collected from all participants. All of them had a chest X-ray taken, and three consecutive sputum samples (early morning collection on 2 consecutive days and one spot collection) were collected for smear, L-J culture, and speciation of NTM.

Sample collection and processing

The procedures of sample collection and sample processing have been published elsewhere.[15] In brief, three consecutive sputum samples were collected and processed by N-acetyl-L-cysteine-sodium citrate-NaOH (NALC-NaOH)[16] method for smear and culture of AFB. The growth was subjected to a rapid immunochromatographic test (ICT, TBC ID, Becton Dickinson, Sparks, MD, USA) for differential identification of MTB as per standard protocol.

Speciation

AFB smear-positive cultures that showed negative ICT results were subjected to speciation by LPA (GenoType Mycobacterium CM/AS, Hain Lifescience, Germany) using H37Rv as positive control. The presence of distinct bands at positions 10, 12, and 13 indicated a positive test for *M. kansasii* isolates.

Drug susceptibility testing

DST of NTM was performed by two different methods. Proportion sensitivity testing (PST) method was done at single concentrations for INH (0.2 µg/ml), RIF (1.0 µg/ml), and EMB (5.0 µG/ml). Broth microdilution method of testing was done using the commercially available SLOMYCO Sensititre system (TREK DIAGNOSTIC Systems Ltd., UK). MICs were established by microdilutions in Mueller–Hinton broth in polystyrene 96-well plates containing lyophilized drugs in doubly increasing concentrations (µg/ml): AMI 1.0 ± 64.0; INH 0.25 ± 8.0; CLA 0.06 ± 64.0; LZD 1.0 ± 64.0; MXF 0.12 ± 8.0; RIF 0.12 ± 8.0; STR 0.5 ± 64.0; SXT 0.12/2.38 ± 8.0/152.0; CIP 0.12 ± 32.0; EMB 0.5 ± 16.0; doxycycline (DOX) 2.0 ± 16.0; RFB 0.25 ± 8.0; and ethionamide 0.3 ± 20.0. Detailed information can be found in the study protocol dx.doi.org/10.17504/protocols.io.nu5dey6. The lowest concentration of the drug that inhibited the visible growth of the isolates tested is defined as Minimum Inhibitory Concentration. The MIC breakpoints of the drugs displaying resistance were interpreted according to the CLSI AM24 guidelines.

Treatment outcome classification

The treatment outcome classification was based on clinical, radiographic, and bacteriological improvement.[10,17]

Favorable outcome

Favorable outcome was defined when a patient is cured with more than two of the following findings: clinical improvement, radiological improvement, and culture negative for three consecutive sputum specimens (sustained for at least 6 months).

Unfavorable outcome

Unfavorable outcome was defined as relapse, failure, default, or death.

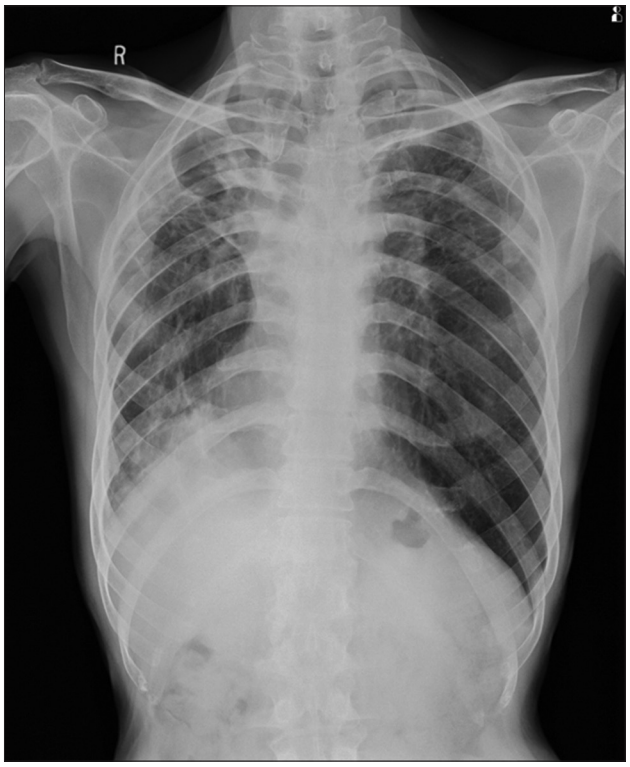


Figure 1: Chest radiograph of a patient at the beginning of treatment with unilateral involvement and two zones

Relapse was defined as isolation of the same mycobacterial species from two cultures, with recurrence of clinical or radiological disease, following a favorable outcome at the end of treatment.

Reinfection was defined as patients who initially had sputum conversion (three consecutive AFB-negative cultures) while on treatment but then subsequently develop positive cultures for NTM by a new strain of NTM, after discontinuing therapy.

Treatment failure was considered if patients have not had response (microbiologic, clinical, or radiographic) after 6 months of appropriate therapy or achieved conversion of sputum to AFB culture negative after 12 months of appropriate therapy.

Default was considered when a patient withdrew from therapy before the prescribed treatment end point or when the outcome at 1 year after treatment was not evaluated.

Death refers a patient who dies due to NTM disease during the course of treatment.

Results

A total of 24 patients (21 male and 3 female patients) were included in this study. The clinical presentation included Cough with expectoration in 21, and hemoptysis in 17 patients. The chest X-ray images revealed bilateral

Table 1: Treatment details of patients with Pulmonary *M. Kansasii* and their DST results by Sensititre method

NTMNO	Treatment regimen	Duration	Change in treatment regimen	Duration	Clinical outcome	Sensititre DST								
						CLARI	RFB	MOX	RIF	SXT	AMI	LZD	CIP	DOX
NT02	RIF, CIP, CLA	36 M	Nil	Nil	Cured	S (2)	S (1)	I (2)	R (2)	S (2;38)	S (4)	I (16)	R (16)	R (>16)
NT05	EMB, INH, RIF	24 M	Nil	nil	Cured	S (0.12)	NG	NG	S (1)	R>8;152	S (4)	S (4)	NG	R (16)
NT08	EMB, INH, RIF	13 days	Nil	Nil	Died	S (2)	S (0.5)	I (2)	R (2)	R (4;76)	S (8)	S (8)	R (8)	R (>16)
NT 12	EMB, INH, RIF	10 M	RIF, CLA, CIP	34 M	Cured	R (>64)	NG	S (1)	S (1)	S (1;19)	S (8)	NG	NG	R (>16)
NT13	EMB, INH, RIF	10 M	RIF, CLA, CIP	7 M	RELAPSE	S (0.5)	NG	R (8)	R (8)	R>8;152	R (>64)	R (64)	R (>16)	R (>16)
NT 15	EMB, INH, RIF	40 M	Nil	Nil	Cured	S (0.12)	NG	NG	R (2)	S (0.5;9.5)	S (2)	NG	R (8)	R (>16)
NT16	EMB, INH, RIF	6 M	RIF, EMB, CLA, CIP	18 M	Cured	S (0.5)	S (0.5)	NG	S (0.5)	S (0.5;9.5)	S (4)	S (2)	S (1)	4
NT27	EMB, INH, RIF	4 M	CLA, CIP, RIF	14 days	Died	S (0.12)	NG	NG	S (0.5)	S (1;19)	S (16)	S (2)	S (1)	R (>16)
NT 28	EMB, INH, RIF	9 M	CLA, RIF, EMB, Septran	24 M	Cured	S (0.25)	NG	S (0.25)	S (0.25)	R (> 8;152)	S (4)	S (4)	I (2)	R (8)
NT 30	EMB, INH, RIF	24 M	Nil	Nil	RELAPSE	R (64)	NG	S (0.25)	NG	S (2;38)	NG	S (2)	S (1)	S (1)
NT 031	EMB, INH, RIF	30 M	Nil	Nil	Cured	S (2)	S (1)	R (4)	R (8)	R (4;76)	S (2)	NG	R (16)	R (>16)
NT 32	EMB, INH, RIF	4 M	RIF, EMB, CLA, CIP	19 M	Cured	S (2)	S (1)	S (1)	R (8)	R (> 8;152)	S (4)	R (64)	R (8)	R (>16)
NT33	EMB, INH, RIF	29 M	Nil	Nil	Cured	S (0.12)	NG	NG	S (0.5)	S (0.25;4.75)	NG	S (2)	S (0.5)	I (4)
NT 35	CLA, INH, RIF	27 M	Nil	Nil	Cured	S (0.12)	NG	I (2)	S (1)	S (1;19)	S (2)	NG	S (1)	I (4)
NT 37	EMB, INH, RIF	13 M	Nil	Nil	Cured	S (0.25)	R (8)	NG	S (0.5)	S (0.12;2.38)	S (16)	S (4)	S (0.5)	R (>16)
NT 43	EMB, INH, RIF	13 M	Nil	Nil	Cured	R (>64)	R (8)	S (0.5)	R (8)	R>8;152	R (64)	S (8)	S (1)	R (>16)
NT46	EMB, INH, RIF	8 M	Nil	Nil	Cured	S (1)	NG	NG	S (0.5)	S (0.5;9.5)	NG	NG	S (1)	I (4)
NT47	EMB, INH, RIF	7 M	Nil	Nil	Cured	S (4)	NG	NG	S (0.5)	S (0.5;9.5)	NG	S (4)	S (0.5)	R (16)

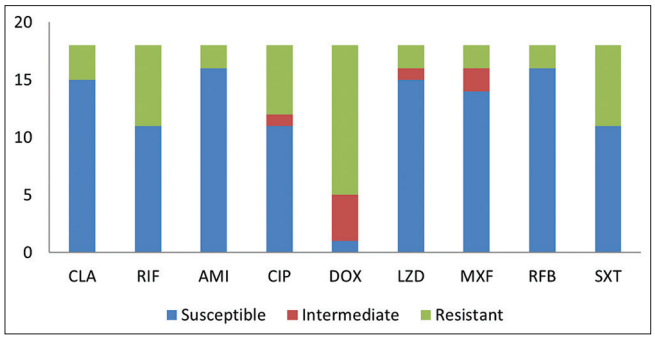


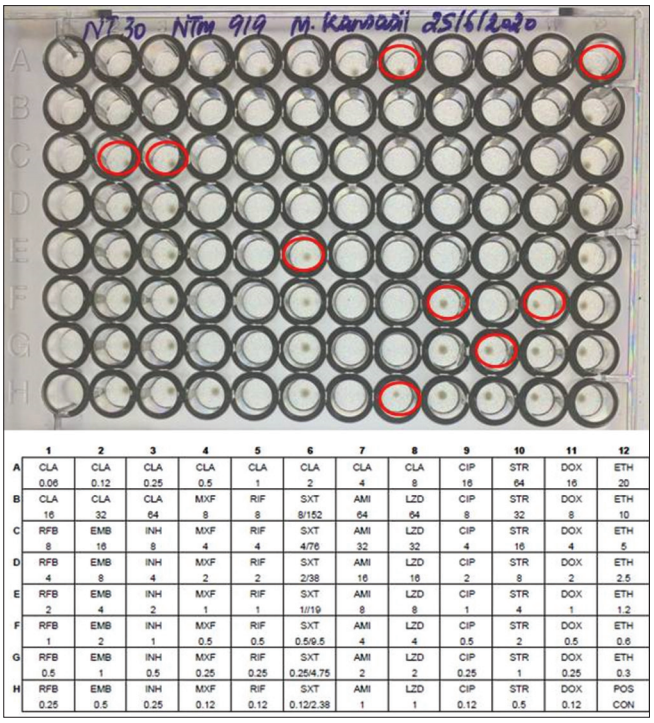
Figure 2: Drug susceptibility testing results of *Mycobacterium kansasii*

parenchymal infiltrates in 19 of the 22 patients. More than three zones were found in 13 patients, out of which 11 had a cavity in a chest X-ray [Figure 1]. Out of 22 patients, prior history of pulmonary TB was found in 21 patients and was treated with anti-TB therapy (ATT) at least once in 6, twice in 9, and thrice in 6 patients. As they were sputum smear positive during the study recruitment, they were all initiated with treatment regimen containing INH, EMB, and RIF. On subsequent identification of NTM species and based on various factors including DST, nine had to undergo regimen change.

A total of 21 and 18 available isolates out of the 22 identified were subjected to DST by PST and Sensititre method, respectively. Drugs that are clinically active against *M. kansasii* were used for therapy that included INH, RIF, and EMB. An alternative thrice-weekly drug regimen with RIF, EMB, and CLA was started in those patients with INH drug resistance. We had used the MIC breakpoints for drugs INH (0.2 µg/ml), RIF (1.0 µg/ml), and EMB (5.0 µg/ml) in the PST method for the isolates initially and published it earlier.[15] The resistance pattern evaluated showed a higher number of patients with INH resistance (20) followed by EMB resistance (10). For extended DST (for antibiotics other than INH, RIF, and EMB), the interpretations from the Sensititre testing method were used. The resistance pattern of the isolates was interpreted using the CLSI M24 guidelines. According to the guidelines, out of the 18 strains of *M. kansasii* subjected to DST, the resistance pattern was demonstrated to be high for DOX (13) followed by RIF and SXT (7) [Figures 2, 3 and Table 1].

Clinical correlation with the drug susceptibility testing

We tried to correlate the patient treatment outcome and the DST patterns of the isolates cultured from them. According to the results by PST method, there were 10 EMB-resistant patients, out of which 8 got cured during the treatment course (12 months of treatment) where 5 patients had to undergo treatment change based on the DST results (CLA, CIP, and RIF). In the remaining 2 EMB-resistant isolates, one died within 1 month of treatment and one was lost to follow-up



	1	2	3	4	5	6	7	8	9	10	11	12
A	CLA 0.06	CLA 0.12	CLA 0.25	CLA 0.5	CLA 1	CLA 2	CLA 4	CLA 8	CIP 16	STR 64	DOX 16	ETH 20
B	CLA 16	CLA 32	CLA 64	MXF 8	RIF 8	SXT 8/152	AMI 64	LZD 64	CIP 8	STR 32	DOX 8	ETH 10
C	RFB 8	EMB 16	INH 8	MXF 4	RIF 4	SXT 4/76	AMI 32	LZD 32	CIP 4	STR 16	DOX 4	ETH 5
D	RFB 4	EMB 8	INH 4	MXF 2	RIF 2	SXT 2/38	AMI 16	LZD 16	CIP 2	STR 8	DOX 2	ETH 2.5
E	RFB 2	EMB 4	INH 2	MXF 1	RIF 1	SXT 1//19	AMI 8	LZD 8	CIP 1	STR 4	DOX 1	ETH 1.2
F	RFB 1	EMB 2	INH 1	MXF 0.5	RIF 0.5	SXT 0.5/9.5	AMI 4	LZD 4	CIP 0.5	STR 2	DOX 0.5	ETH 0.6
G	RFB 0.5	EMB 1	INH 0.5	MXF 0.25	RIF 0.25	SXT 0.25/4.75	AMI 2	LZD 2	CIP 0.25	STR 1	DOX 0.25	ETH 0.3
H	RFB 0.25	EMB 0.5	INH 0.25	MXF 0.12	RIF 0.12	SXT 0.12/2.38	AMI 1	LZD 1	CIP 0.12	STR 0.5	DOX 0.12	POS CON

Figure 3: Sensititre plate layout with Minimum inhibitory concentration (MIC) values marked in red

during treatment. During the follow-up of the 8 cured patients, one of them got relapsed at 17th month with *M. kansasii* infection. Similarly, out of 20 INH-resistant isolates, 16 of them got cured with treatment change for 7 patients (CLA, CIP, and RIF) during the treatment course. However, two of them got relapsed at 17th and 23rd month of follow-up. In the remaining 4 INH-resistant isolates, 2 died, 1 was lost to follow-up, and 1 was treatment failure.

In extended DST with Sensititre method, three patients were resistant to CLA, in which one patient's treatment regimen was changed from INH, RIF, and EMB to CLA, CIP, and RIF at the 10th month of treatment. However, the patient got successfully cured indicating minimal role of CLA resistance in the treatment outcome. Two other patients who got cured had no treatment change and hence could not correlate it with CLA resistance. A total of eight patients were treated with CIP in a combination of CLA, RIF, and/or EMB as part of changed regimen, and for one patient, the treatment was initiated with these drugs in the initial phase itself. Out of 8 patients with CIP therapy, 3 patients demonstrated resistance to CIP, out of which 1 died and 2 got cured during the treatment course but got relapsed at 17th and 23rd month.

The RIF resistance when tested by PST was limited to only one patient, but seven patients were resistant by Sensititre method, out of which 1 died, 1 got relapsed, and 5 got cured. Resistance toward MOX, DOX, AMI, LZD, and SXT was also seen in the Sensititre DST.

However, none of the patients were treated with these antibiotics, and hence, we could not correlate them with the clinical outcome.

Discussion

This study aimed to get a broader perspective toward the DST of *M. kansasii* strains and correlate them with the treatment outcome. Patients included in this study showed extensive lung damage at the time of NTM diagnosis, indicating a delay in diagnosis or misdiagnosis as pulmonary MTB infection earlier and treated with ATT. Only after completion of at least one course of anti-TB treatment, the patients were suspected of having NTM and referred to a higher center. This could be mainly due to the nonavailability of protocols to diagnose pulmonary NTM in the standard of care guidelines, earlier in the course of respiratory illness. Irrespective of a wide variety of drug availability, first-line treatment of *M. kansasii* is limited to INH, EMB, and RIF. The poor correlation of the INH resistance with the clinical outcome can be explained by the critical concentration (0.2 μg/ml) we used which is generally not recommended for *M. kansasii*. This could have resulted in a false interpretation of INH resistance because the MIC values usually ranges from 0.5 to 5 μg/ml for *M. kansasii*.[18] This issue was further illustrated by Heifets' work in which the 100 *M. kansasii* isolates when evaluated by agar dilution methodology, almost all, were completely resistant to 0.2 μg/ml of INH and susceptible or partially resistant to 1 μg/ml of INH.[19]

The clinical MIC cutoff for EMB to define susceptibility/resistance against *M. kansasii* is generally prescribed as 4 μg/ml. However, there is increasing evidence that by this definition, many *M. kansasii* isolates are resistant to EMB.[20] In our study, we had used the critical concentration of 5 μg/ml in the PST method of testing and reported 10 EMB-resistant isolates out of which one died and one was lost to follow-up. Some of the EMB-resistant patients getting cured can be explained by the evidence of pronounced synergism of other drugs when combined with EMB. For example, the strains resistant to CIP and EMB when tested separately have shown susceptibility to the combination of these drugs.[21]

Given the importance of RIF to the current regimen, the ATS/IDSA guidelines recommend routine susceptibility testing of RIF alone.[10] It also recommends that only in the presence of RIF resistance extended susceptibility testing should be employed. However, recently, CLA and fluoroquinolones have been promoted as effective therapies, although there is some concern about rising resistance to the latter, including CIP and MOX.[18] In instances of RIF-resistant *M. kansasii*, the ATS/IDSA recommends a combination of CLA, MOX, and a third agent with *in vitro* susceptibilities such as EMB or SXT.[10] In our study, we found three RIF-resistant isolates of whom 2 were also resistant to CIP and 2 were resistant to MOX. Further studies will be needed to elucidate the clinical significance of such combined drug resistance patterns and their role in NTM treatment. In addition to the resistance patterns elucidated in this study, the role of earlier ATT treatment in resistance emergence should also be considered.

In cases of the resistance pattern demonstrated with the CLSI-recommended drugs, other drugs showing susceptibility shall be considered. For example, data from China demonstrated AMK to be an active agent against *M. kansasii in vitro*,[22] which is similar to our study where 16 out of 18 isolates tested were susceptible to AMK. When the CLA resistance pattern was reviewed, three of the resistance isolates in our study demonstrated the values to be ≥64 μg/ml. Studies conducted in Brazil,[23] Taiwan,[20] and Iran[23] established that all cultures of *M. kansasii* were susceptible to CLA with the lower MIC values ranging from 0.12 to 8 μg/ml. However, a study from China demonstrated the MIC90 value to be 128 μg/ml. This indicates increased concentration of the MICS for CLA testing.[22] Similarly, most of the isolates in our study were also susceptible to LZD which could be another drug of choice. Studies have demonstrated the improved activity of LZD, CLA, and MOX in a short course or intermittent therapy of lung disease.[24]

Conclusion

NTM diagnosis, if made early along with appropriate species identification in a symptomatic patient with lesions on chest X-ray, sputum AFB smear positive and but Genxpert negative, appropriate treatment could be initiated. Simultaneous DST by Sensititre method with various concentrations of the drug will be useful for analyzing the drug resistance since the NTM treatment is different from ATT. Further, DST with newer drug results such as bedaquiline and delamanid shall be initiated since it will be useful for treating the first-line and second-line drug-resistant isolates. However, large-scale studies are needed for demonstrating the drug susceptibility pattern of NTM in various parts of the country.

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Conflicts of interest

There are no conflicts of interest.

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