



An Update on the Prevalence of Nontuberculous in Clinical Samples in Iran during 2000-2022: A Retrospective Systematic Review and Meta-Analysis

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ABSTRACT

Background and objectives: Nontuberculous mycobacteria (NTM) are isolated from domestic and animal products as well as man-made systems such as medical devices, drinking water systems, water tanks, and shower streams. This study aimed to investigate the prevalence of NTM in clinical samples in Iran during 2000-2022.

Methods: Published studies addressing the prevalence of NTM in clinical samples in Iran were reviewed according to the Preferred Reporting Items for Meta-Analyses and Systematic Reviews protocol. Original articles in Persian and English published between January 2000 and 2022 in databases such as Scopus, PubMed, Web of Science, Google Scholar, and Iranian databases were included. The prevalence of NTM at 95% confidence interval (CI) was calculated by comprehensive meta-analysis.

Results: Overall, 26 studies were included in the review. The combined prevalence of NTM in positive mycobacterial cultures was 4.5% (95% CI: 3.1-6.5). *Mycobacterium simiae* [35.8% (95% CI 16.4-44.4)], *Mycobacterium intracellulare* [19% (95% CI 8.7-28.3)], and *Mycobacterium kansasii* [13.4% (95% CI 7.3-24.3)] were the most common slowly growing species, while *Mycobacterium fortuitum* [24.6% (95% CI 12.9-46.7)], *Mycobacterium terrae* [18.5 % (95% CI 11.5-29.2)], and *Mycobacterium gastri* [15.9% (95% CI 6.0-41.2)] were the most prevalent rapidly growing mycobacteria.

Conclusion: In summary, our findings indicate a relatively high combined prevalence of NTM in clinical samples in Iran. Some of these species such as *M. simiae* can have clinical and radiologic manifestations similar to those of TB and are resistant to anti-TB drugs. Therefore, standardizing the use of molecular methods for the detection of NTM seems necessary.

Keywords: [Nontuberculous Mycobacteria](#), [Mycobacterium abscessus](#), [Meta-Analysis](#).

INTRODUCTION

Nontuberculosis mycobacteria (NTM) are described as mycobacterial pathogens other than *Mycobacterium tuberculosis* (MOTT) and *Mycobacterium leprae* strains. They are a heterogeneous group of bacteria that cause a fundamental but frequently unvalued global burden of disease (1, 2). These ubiquitous bacteria have a high prevalence in the environment. There is ample evidence that these microorganisms originate from the environment. In the 1980s, NTM was identified as a human pathogen (3, 4). Although most NTM are saprophytes, one-third of them are related to human diseases (5). Generally, most NTM are aerobic, immotile bacteria with a firm and dense cell wall (6). The thickness of NTM cell wall functions as a natural protective shield against disinfectants and antibiotics (7). Therefore, NTM grow in most environments around humans. The increasing rate of infections caused by NTMs may be related to the presence of NTMs in domestic and animal products, medical devices, drinking water systems, water tanks, and shower streams (8).

Infections caused by NTM are relatively uncommon and often reported in immunocompromised persons (9). Certain features of NTM are similar to *M. tuberculosis* that make NTM difficult to differentiate (10). Nevertheless, NTM usually do not respond to common tuberculosis (TB) drug regimens, causing misdiagnosis and poor treatment, especially in low-resource settings (11). Current evidence advises that diseases resulting from NTM are much more prevalent globally than previously believed, and possibly rising in frequency worldwide (12). A report from Canada showed that the incidence of NTM was 150,000 cases per year (13).

In 1959, Ernest Runyon classified NTM based on growth rates, colony morphology, and pigmentation (14). Accordingly, NTM were categorized into four groups: rapid growers (groups I to III) and slow growers (group IV) (15, 16). Slowly growing species (SGM) typically require more than 7 days before colonies become visible on solid media, while rapidly growing species (RGM) form colonies on selective media within 2–5 days (17). These organisms cause four distinct clinical diseases, including progressive pulmonary disease, superficial lymphadenitis, disseminated

The subject of NTM is particularly troubling in developing countries owing to limited published information and unsuitable identification. Meta-analysis studies on the prevalence of NTM have been previously conducted in Iran. Given that the last meta-analysis on this subject dates back to 2016 (18), this study aimed to investigate the prevalence of NTM in clinical samples during 2000-2022.

MATERIALS AND METHODS

This systematic review and meta-analysis was conducted by reviewing published studies on the prevalence of NTM among clinical samples in Iran. The study was carried out according to Preferred Reporting Items for Meta-Analyses and Systematic Reviews (PRISMA) protocol.

The search was performed only for original cross-sectional studies in Persian and English that have been published between January 2000 and 2022 in international electronic databases, such as Scopus, PubMed, Web of Science, Google Scholar, and Scientific Information Database, IranMedex, Magiran, and IranDoc. The search process was according to the combination of Medical Subject Headings (MeSH) text words such as “non-tuberculosis mycobacteria”, “NTM”, “MOTT”, “atypical mycobacterium”, “RGM”, “SGM”, and “Iran”.

As an example among the different databases, the search strategy strings in PubMed are summarized as follows; Non-tuberculosis Mycobacteria (MeSH Terms) OR atypical mycobacterium (MeSH Terms) OR NTM (Title/Abstract), MOTT (MeSH Terms) AND (rapid-growing mycobacterium (MeSH Terms) OR RGM (Title/Abstract)), AND (slow-growing Mycobacterium (MeSH Terms), OR SGM (Title/Abstract)). All searches were performed in Persian databases with Persian equivalent words with the same strategy. In addition, the reference section of the original and review studies was screened to find further articles for inclusion in the present systematic review and meta-analysis. All of these searches have completed by two researchers individually.

Duplicates were initially identified and eliminated after entering all the recognized studies into a self-created database. After that,

the articles were assessed by two reviewers (AF and AS) by screening titles, abstracts, topics, and finally full texts. At each level, the reviewers independently screened the articles and finally merged their conclusions. Discrepancies were resolved by discussion before finalizing the records for the next level. In case of disagreements, a third assessor was assigned to make a decision. Finally, the studies were assessed for eligibility before the final selection.

We included studies that met the following eligible inclusion criteria: (1) original data, (2) studies on the prevalence of NTM, and (3) studies with accepted standard methods including growth in Lowenstein-Jensen media containing p-nitrobenzoate or thiophene-carboxylic acid hydrazide, growth rate, pigment production, growth at 42 °C and 44 °C, tellurite reduction, arylsulfatase activity, tween hydrolysis, nitrate reduction, catalase, urease, tolerance to the NaCl 5%, and molecular methods such as PCR-RFLP (*PRA hsp65*), sequencing of *hsp*, PCR and sequencing of *16s rRNA*, multiplex allele-specific PCR (MAS-PCR), Line Probe Assay (LPA), PCR and sequencing *rpoB* gene, sequencing *erm* gene, multilocus sequence analysis of *16S rRNA*, *rpoB*, and *ITS* genes. Reviews, case reports, and conference abstracts were excluded. , studies not performed according to the accepted standard methods.

The studies' quality was assessed using the criteria specified in Critical Appraisal Skills Programmed checklists (www.casp-UK) (19). This assessment is based on answers to 10 questions designed for each study. If any query data was available, the answer was 'yes'. In case of doubt or lack of appropriate answer, the answer was 'no' or 'cannot tell'. Based on the number of questions answered "yes", the studies were classified into three categories: good (score of 8-10), moderate (score of 6-8), and poor (score of <6) (20). Finally, weakly scored studies were not enrolled in the study.

In this review, two researchers independently extracted the data including the first author, study's time, publication time, geographic location, NTM, methods, and mean age of patients. Meta-analysis was conducted for determining the prevalence of NTM at 95% confidence interval (CI) by comprehensive meta-analysis (V2.0, Biostat, Englewood, NJ, USA).

Random effect model was used and tested with Cochran's Q test and I^2 to determine the possibility of heterogeneity between studies. Egger weighted regression test was applied for the statistical assessment of publication bias, and *p*-values less than 0.05 were considered statistically significant. In addition, funnel plot was used to evaluate publication bias in the studies.

RESULTS

As shown in [figure 1](#), 1,078 articles were retrieved through database searches. After excluding 452 duplicate articles, 626 studies were assessed, 201 of which were removed because of title or abstract irrelevance. Next, 425 full texts were evaluated for content and method. Finally, 26 eligible studies were systematically reviewed and analyzed.

The characteristics of the included studies are summarized in [table 1](#). The mean age of patients positive for NTM was between 11 and 80 years. Geographic locations included Tehran, Kashan, Khuzestan, Tabriz, Yazd, Golestan, Kermanshah, Mashhad, and Hormozgan ([Table 1](#)).

All included studies used conventional methods for the detection of mycobacteria. These methods were growth in Lowenstein-Jensen media containing p-nitrobenzoate or thiophene-carboxylic acid hydrazide, growth rate, pigment production, growth at 42 °C and 44 °C, tellurite reduction, arylsulfatase activity, tween hydrolysis, nitrate reduction, catalase, urease, and tolerance to the NaCl 5%. The majority of NTM were isolated from respiratory and bronchoalveolar lavage samples. Our review showed that the prevalence of NTMs in positive mycobacterial cultures varied from 0.1 to 72.7%. As shown in [table 2](#) and [figure 2](#), the combined prevalence of NTM in clinical samples was 4.5% (95% CI: 3.1-6.5, $Q = 1562.7$, $Z = 15.2$, $I^2 = 98.4$, and $p=0.00$). According to funnel plot, publication bias was visually found among the included studies ([Figure 3](#)). Egger's weighted regression test results also suggested the presence of bias in the studies ($p=0.6$). Therefore, there is a possibility of publication bias due to the existence of small studies included in this review. As reported in [table 2](#), the most common SGM among NTM species were *Mycobacterium simiae* [35.8% (95% CI 16.4-44.4)],

Mycobacterium intracellulare [19% (95% CI 8.7-28.3)], and *Mycobacterium kansasii* [13.4% (95% CI 7.3-24.3)], while *Mycobacterium fortuitum* [24.6% (95% CI

12.9-46.7)], *Mycobacterium terrae* [18.5 % (95% CI 11.5-29.2)], and *Mycobacterium gastri* [15.9%(95% CI6.0-41.2)] were the most prevalent RGM among NTM species.

Table 1-Characteristics of the studies included in the review

First author (reference)	Time of study	Date of publication	Location	Sample size	NTM Number (%)	Identification methods	Mean age of patient (years)
Derakhshani Nejad(40)	2003-11	2014	Tehran	8322	124	Conventional tests, PCR-RFLP	57 ±18.9
Heidari(41)	2007-8	2009	Tehran	371	43	Conventional tests, PCR-RFLP	14-80
Nasiri(42)	2010-12	2014	Tehran	6426	9	Conventional tests, sequencing	11-80
Javid(43)	2007- 8	2009	Golestan	104	17	Conventional tests, sequencing	14 ≤65
Shafipour(44)	2010-11	2013	Golestan	336	16	Conventional tests	44 ±23.3
Moghtaderi(45)	2000-10	2011	Tabriz	235	15	Conventional tests	-
Heidar Nejad(46)	2001	2001	Tabriz	165	10	Conventional tests	44.01±18.23
Naserpour Farivar(47)	2002- 4	2006	Sistan-Baluchestan	210	59	Conventional tests	20 ≤60
Naderi(48)	2003- 4	2006	Sistan-Blochestan	150	20	Conventional tests	-
Namaei (49)	2002	2003	R.Khorasan	1700	8	Conventional tests	-
Hashemi-Shahraki (50)	2008-12	2014	Khuzestan	2313	92	Conventional tests, sequencing	-
Hashemi-Shahraki(51)	2009-12	2013	khuzestan	190	23	Conventional tests, sequencing	48.3-57.1
Khosravi(52)	2007-8	2009	Khuzestan	150	8	Conventional tests PCR-RFLP	24-36
Yazdi(53)	2009-10	2012	Yazd	32	1	Conventional tests	-
Zilae(54)	2012-15	2016	Kashan	106	4	PRA <i>hsp65</i>	-
Nour-Neamatollahie(55)	2011-13	2017	Tehran	10,377	59	PCR-RFLP (PRA <i>hsp65</i>)	50.9 ± 7.6
Nasiri(56)	2014-16	2018	Tehran	410	56	PCR-RFLP (PRA <i>hsp65</i>)	50.9 ± 7.6
Nasiri(57)	2016-17	2018	Tehran	230	12	<i>hsp 65</i> - PRA, sequencing of 16S rRNA, <i>rpoB</i> , and ITS genes	51.4
Irandoost(58)	2014-16	2018	Tehran	6800	64	PRA and sequencing of <i>hsp65</i>	-
Aghajani(59)	2011-19	2019	Tehran	15829	591	<i>hsp65</i> - PRA, sequencing <i>16S rRNA</i> , <i>rpoB</i>	50.7 ±18.4
Mortazavi(60)	2015-17	2019	Tehran	478	53	<i>hsp65</i> -PRA, sequencing <i>16S rRNA</i> , <i>rpoB</i>	43.4 ±15.7
Davari(61)	2013-15	2018	Tehran	520	61	Multilocus sequence analysis of 16S rRNA, <i>2rpoB</i> , and ITS genes	49.6 ± 16.6
Karami-Zarandi(62)	2017-19	2019	Tehran	5061	60	LPA, PCR and sequencing <i>16s rRNA</i>	58.3±18.3
Khosravi(63)	2016-18	2018	Khuzestan Kermanshah Hormozgan	55	40	PCR and sequencing <i>rpoB</i> gene, sequencing <i>erm</i> gene	47.4 ±19.9
Ayoubi(64)	2011- 18	2021	Tehran	15771	658	(RFLP)-PCR of a <i>hsp65</i> fragment, Nested-PCR	-
Shafipour(65)	2016-18	2021	Gorgan	2994	12	Conventional tests, PCR(16S rRNA gene)	59.9 ± 16.9

Table 2-Overall effects and combined prevalence of NTM

Subgroups	Number of studies	Heterogeneity test			Egger's test			Random model	
		Prevalence (95% CI)	Z	p	Q	p	I	t	p
Combined NTM	26	4.5(3.1-6.5)	15.2	0.00	1562.7	0.00	98.4	0.5	0.6
Slowly growing mycobacteria									
<i>M. simiae</i>	25	35.8(16.4-44.4)	2.5	0.01	102.3	0.00	93.1	3.1	0.01
<i>M. kansasii</i>	22	13.4(7.3-24.3)	5.1	0.00	64.1	0.00	87.5	0.6	0.5
<i>M. goodnae</i>	13	6.6(0.6-17.5)	3.6	0.00	31.5	0.00	90.4	1.4	0.27
<i>M. intracellulare</i>	13	19(8.7-28.3)	17.4	0.00	2.7	0.4	0.00	2.2	0.15
<i>M. avium complex</i>	12	10.3(1.6-18.1)	14.8	0.00	1.7	0.45	0.00	0.65	0.63
<i>M. szulgai</i>	23	9.1 (3.2-28.1)	2.1	0.00	1.1	0.00	0.00	0.00	0.03
Rapid growing mycobacteria									
<i>M. fortuitum</i>	24	24.6(12.9-46.7)	2.2	0.02	152.3	0.00	94	2.1	0.06
<i>M. abscessus</i>	12	10.6(4.3-11.8)	9.1	0.00	2.1	0.00	0.00	1	0.31
<i>M. chelonae</i>	11	6.8(3.8-11.7)	10.7	0.00	2.2	0.31	12.4	1	0.01
<i>M. thermoresistibile</i>	10	2.95(1.4-8.1)	7.2	0.00	0.76	0.00	0.00	0.00	0.00
<i>M. terrae</i>	19	18.5 (11.5-29.2)	8.1	0.00	0.00	0.00	0.00	0.00	0.00
<i>M. gastri</i>	23	15.9 (6.0-41.2)	6.4	0.00	1.4	0.00	0.00	0.00	0.00

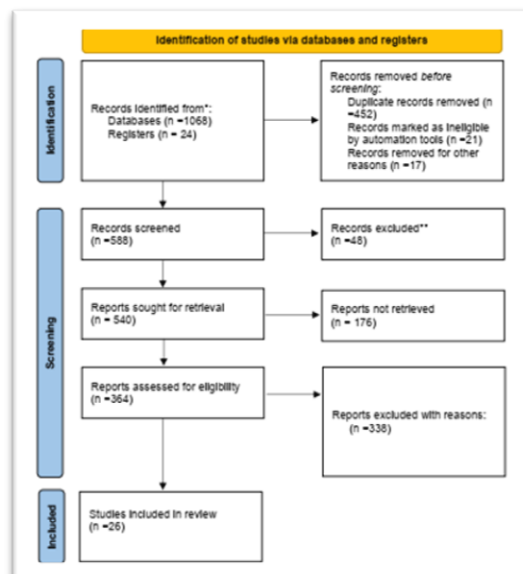


Figure 1- Flow diagram of the study process

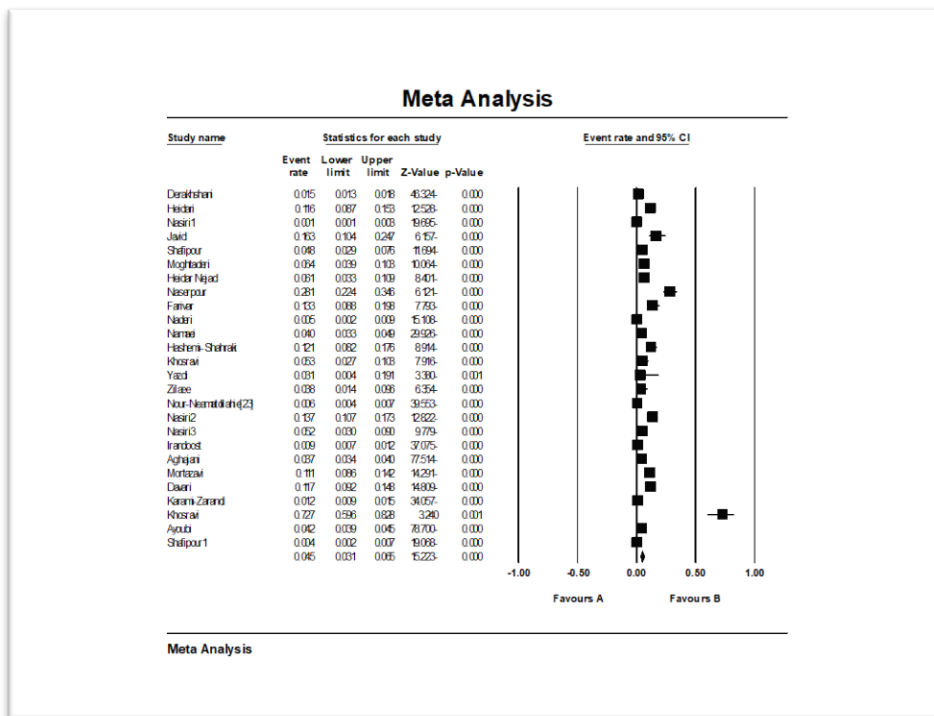


Figure 2- Forest plot of the meta-analysis of epidemiology of NTM in clinical samples from Iran

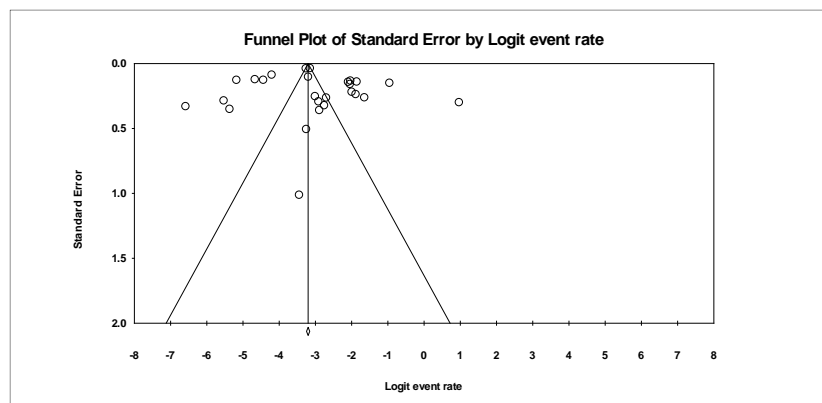


Figure 3-Funnel plot of the meta-analysis of epidemiology of NTM in clinical samples from Iran

DISCUSSION

Since many studies do not consider infections caused by NTMs as a public health problem, there is not enough data about these microorganisms and their frequency distribution, at least in Middle Eastern and third-world countries. This has made developing infection control strategies challenging (21).

Our review showed that the prevalence of NTM in clinical samples varied from 0.1 to 72.7%. As mentioned in the results, the majority of NTM were isolated from respiratory and bronchoalveolar lavage samples. These findings emphasize the importance of identifying NTM from suspected pulmonary TB patients (22).

In line with our findings, a study from Saudi Arabia reported that pulmonary (54.7%) and bronchial lavage/wash (22.1%) specimens were predominant (23). The difference in the prevalence of NTM in the studies reviewed in our survey might be due to the difference in the molecular techniques used in each study, the geographic region, types of clinical specimens, laboratory personnel skills, sanitation, and living conditions (4).

We showed that the combined prevalence of NTM isolated from clinical samples in Iran was 4.5% during 2000-2022. Because the manifestations of NTM and TB are similar and all NTM are acid-fast and cannot be segregated by phenotypic methods, NTM may

be mistaken for TB. Moreover, diseases caused by NTM typically do not respond to anti-TB drugs (24). Furthermore, in some cases, patients with multi-drug resistant TB were in fact infected with NTM (25).

Reports should be interpreted with caution because it is often challenging to determine whether NTM are the real source of infection (1). In line with our results, the study from Saudi Arabia reported a prevalence rate of 1.4% for NTM (23). Studies by Pokam et al. (12) and Aliyu et al. (11) in Nigeria reported prevalence rates of 16.5% and 15%, respectively. However, higher prevalence rates were reported in studies from Canada (33%) and the Netherlands (25%) (26, 27). A systematic review and meta-analysis published by Nasiri et al. in 2015 reported a pooled prevalence rate of 10.2% in Iran, which is higher than the rate found in our study (11.2%). This could be related to the source of NTM because our study was focused on clinical samples, but the mentioned study was focused on suspected TB patients (18). In recent years, the reports of NTM have been rising, mainly because of the active search for NTM species, improvements in culture methods (28), and most importantly, the use of sensitive molecular techniques (22, 29). Here, we detected a combined prevalence rate of 4.5% in clinical specimens, which is similar to the rate reported by Khaledi et al. in Iran in 2016 (2). Subgroup analysis in our review showed that the combined prevalence of *M. simiae* (35.8%), *M. intracellulare* (19%), and *M. kansasii* (13.4%) was highest among SGM, while *M. fortuitum* (24.6%), *M. terrae* (18.5%), and *M. gastri* (15.9%) were the most prevalent RGM. Evidence suggests that RGM species are among the most predominant NTM associated with nosocomial infections. As described by previous reports, tap water, dialysis water provided from tap water, drinking water, shower water, and piped water systems in clinical settings are the common sources of NTM-related nosocomial infections (30). In addition, RGM are relatively resistant to standard disinfectants such as chlorine, alkaline glutaraldehydes, and antimicrobial agents compared to *M. tuberculosis*; thus, their eradication is more difficult (31). In line with our study, a previous review on the distribution of NTM species among environmental and clinical samples in the Middle East reported that 58.7% of isolates

were SGM and 41.2% were RGM. This study also reported similar prevalence rates for SGM (56.4%) and RGM (44.6%) in Iran (21). Moreover, this study reported *M. fortuitum* (60.1%) as the most prevalent RGM isolated from clinical specimens in the Middle East (30). *M. fortuitum* was detected in 71.9%, 54.4%, 46.6%, and 48.9% of RGM isolates from Iran, Saudi Arabia, Turkey, and Pakistan, respectively (6). Other reports from Iran's neighboring countries (Saudi Arabia and Kuwait) also found *M. fortuitum* as the predominant isolate (23, 32). The proportion of RGM in pulmonary diseases from Iran and other Asian countries is much higher than in European and North American countries (18, 22, 33). For example, studies from the Netherlands and the United States reported a prevalence rate of 3% and 5% for RGM, respectively (26, 34).

In our study, *M. simiae* was found as the most predominant SGM among NTM isolates. This finding is in agreement with the results of previous reviews from Iran (18, 22). On the contrary, in developed countries, *M. avium* complex has been described as the most common NTM species (35). It is noteworthy to mention that *M. simiae* is an endemic SGM in Iran. It is often not distinguishable from TB complex due to its similar clinical and radiologic manifestations as well as the lack of response to anti-TB drugs (36). Therefore, it is recommended to consider *M. simiae* in cases where anti-TB treatment does not respond (37). The spread of diseases caused by mycobacteria, especially respiratory diseases, and the possible inappropriate treatment imposes a lot of costs on both patients and health systems. Nevertheless, most laboratories do not have accurate diagnostic criteria for NTM owing to the lack of appropriate equipment and qualified experts (1). In recent studies, the increased use of molecular methods has increased the accuracy of NTM diagnosis (38). National TB reference laboratories necessitate standardizing existing protocols for the identification of NTM in Middle Eastern countries (21, 39). Thus, given the rising importance of NTM, quick and precise identification of NTM is of great importance for active management strategy against NTM Infections (21). The main limitation of the present study was that studies published in languages other than English and Persian have not been included in the analysis.

Another limitation was that the protocol of this systematic review and meta-analysis was not registered in a standard platform like Cochrane or Prospero.

CONCLUSION

In summary, our findings indicate a relatively high combined prevalence of NTM in clinical samples in Iran. Some of these species such as *M. simiae* can have clinical and radiologic manifestations similar to those of TB and are resistant to anti-TB drugs. Therefore, standardizing the use of molecular methods for the detection of NTM seems necessary.

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Ethics approvals and consent to participate

Not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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