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Prevalence of Nontuberculous Mycobacteria (NTM) in Iranian Clinical Specimens: Systematic Review and Meta-Analysis

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ABSTRACT

***Background*:** Although, nontuberculous mycobacteria can cause disease in different organisms, they usually are not reported in most countries because scientists in general consider them as non-pathogens. But, increasing nontuberculous mycobacteria diseases occurrence has changed this belief. Nevertheless, there is no meta-analysis review about prevalence of nontuberculous mycobacteria in Iran.

***Methods*:** Any data about prevalence of nontuberculous mycobacteria in clinical specimens in Iran were retrieved by searching data bases such as Pub Med, MEDLINE, and Iranian data bases. Then the meta-analysis was performed by comprehensive meta-analysis software (CMA).

***Results*:** The meta-analysis showed that the prevalence of nontuberculous mycobacteria in the clinical specimens in Iran was 1.3%. In the studies that had sample size less than 300, and in studies conducted after 2004, the prevalence was higher. Also, the prevalence of nontuberculous mycobacteria was higher in the West of Iran. In this study, the most prevalent rapid-growing mycobacterium was *Mycobacterium. fortuitum* and most prevalent slow-growing mycobacterium was *M. simiae* with the prevalence 44.2% and 14.3%, respectively.

***Conclusion*:** *M. simiae* is the most prevalent nontuberculous mycobacteria in the clinical specimens in Iran. As this species of nontuberculous mycobacteria has similar clinical and radiological manifestations with tuberculosis, it is often treated as tuberculosis. Unfortunately, *M. simiae* is resistant against first-line anti-TB drugs resulting in treatment failure after using routine anti-TB medication. Therefore, there is an urgent need for application of new diagnostic strategy for identification of nontuberculous mycobacteria species.

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Introduction

Mycobacteria are genus of bacteria which can cause different diseases in human, apart from TB complex species, there are other species that called nontuberculous mycobacteria (NTM) and they are important in medical microbiology (1). According to the Runyon's classification, NTM are classified based on growth rates and production of pigment. Groups I to III are slow-growing NTM, and group IV are rapid growing (2). The slow-growing NTM are subdivided into group I photochromogens (producers of pigment in the vicinity of light), group II scotochromogens (producers of pigment in the lack of light), and group III nonchromogen (2).

A few species of rapidly growing mycobacteria (RGM) such as *Mycobacterium fortuitum* group, the *M. chelonae/abscessus* group, and the *M. smegmatis* group are able to produce diseases in humans. The acid-fast property, growth of easily visible colonies through 7 days on solid media, aryl sulfatase activity and the lack of any pigmentation are main characteristics for recognition of them (3). In the recent years, infections result from these rapidly growing NTMs has been reported as complications of surgical procedures (4, 5).

The important members of slow-growing NTM are *M. avium* complex (which contain *M. avium* and *M. intracellular*) which are present in all natural habitats. *M. avium* complex have been isolated from various sources, for example, water, soil, air, plants and also from animals (5).

Many infections such as skin infections, cervical lymphadenitis, and joint infections, pulmonary infections, and nosocomial infections, bacteraemia are caused by NTM (6). NTM diseases are not reported in most countries because health managers have encountered with more threatening health problems, thus, NTM have not been regarded as public health concern (7). But, during recent years, increasing occurrence of diseases caused by NTM has been reported from many locations, for example, southwestern Ireland, many countries in Asia, Australia, New Zealand and Canada (7). In Australia, the incidence of NTM disease has increased from 2.2 to 3.2 per 100,000 populations between 1999 and 2005. Interestingly during this time, the influenced population changed from middle-aged men who smoked to aged nonsmoking women (8). This change has been attributed, partly, due to improved detection techniques, along with greater disease awareness and an actual increase in disease prevalence (7). Recently, based on the studies carried out in Canada, the estimate of pulmonary diseases caused by NTM was at least 150,000 cases in year and U.S experts believe that the frequency in some fields is at least ten times greater than *Mycobacterium tuberculosis* (9). In general, there is little information about the prevalence of NTM in Iran, so the aim of this study was to characterize the prevalence of NTM in Iranian clinical specimens using a meta-analysis based on the principles of standard methods for analysis (10).

Methods

Search strategies

A database was built for prevalence of NTM in Iran for articles that were published up to November 2014 using Pub Med, Web of Science, Scopus, MEDLINE, EMBASE, Cochrane Library, Google Scholar, Science Direct, Iran Medex, and the Scientific Information Database. The search was limited to original literatures published in English and Persian that presented the prevalence, incidence or distribution of NTM in Iran. The keywords and terms such as, nontuberculous mycobacteria, NTM, NTM infections or NTM diseases, prevalence, incidence, distribution, study and Iran from Mesh or medical abstracts were used for searching. Similarly, the searching was performed with same strategies and relevant Persian keywords among Iranian databases. We searched Iranmedex (www.iranmedex.com), Scientific Information Database (www.sid.ir), Magiran (www.Magiran.com), Irandoc (www.irandoc.ac.ir), and Iranian National Library (www. nlai.ir), Civilica

(www.civilica.com), Pmdr (www.pmdr.ir). Also, references from retrieved papers in English and Persian were checked for additional data. We recruited only full text articles, not any meeting or conference abstracts and case reports.

Two investigators independently searched the electronic databases with the identical method. The titles, abstracts and full texts were reviewed independently by two reviewers to determine if they met eligibility criteria for inclusion. References in the studies were reviewed to explore additional papers.

Inclusion and exclusion criteria

We focused on original articles presenting cross-sectional or cohort studies on the prevalence of NTM. The papers with sample size greater than 50 which reported the prevalence of NTM up to November 2014 were included in our study. Review articles, animal studies, congress and meeting abstracts, articles reported in languages other than English or Persian, meta-analysis or systematic reviews, duplicate publications of the same study, articles available only in abstract form, case report articles were excluded. Two reviewers independently completed this course in order to reduce the risk of errors.

Data extraction

We designed a data abstraction form for our reviewers. The following data were included in our forms (e.g., the first authors' names, time of study, year of publications, location of participants, characteristics of participants (e.g., age, sample size) and the prevalence of NTM.

Statistical analysis

Analysis was performed by Comprehensive Meta-Analysis Software Version 2.0 (Biostat, Englewood, NJ). Prevalence was reported by 95% confidence intervals (CIs). Random effect model was used for meta-analysis as well as to take into account the possibility of heterogeneity between studies, which was tested with the Cochrane Q test and I2 test. To evaluate possible publication bias, Egger weighted regression method was used. ($P<0.05$ was considered indicative of a statistically significant publication bias).

Results

Characteristics of selected studies

The process of study selection is shown in figure 1. Briefly, at first by use of multiple databases in English and Persian, 131 potentially relevant articles identified. 42 articles were excluded because of duplications, and 27 papers were removed, due to the irrelevant titles. Then on the basis of abstract evaluation, 26 articles were excluded (18 article topics were not relevant and 8 articles were case reports). At this stage 35 full text articles evaluated and 17 articles were excluded after full text evaluation (14 due to lack of data availability and 3 did not reported NTM prevalence data). Finally 18 selected articles were recruited in our meta-analysis.

The properties of included articles are summarized in table I. The age of patients was 11 up to 85 years old. Most of studies were done in the center of Iran. As shown in table II, among these 18 articles from five locations of Iran, 6 (33.3%) were reported from center of Iran (5 from Tehran, 1 from Isfahan province), 4 (22.2%) reported from south (all of them from Khuzestan province), 3 (16.6%) reported from east (2 from Sistan-Baluchestan province and 1 from Razavi Khorasan province), 2 (11.1%) reported from west (all of them from East Azarbaijan province), and finally 3 (16.6%) were reported from North (1 from Mazandaran province and 2 from Golestan province). The prevalence of NTM in different parts of Iran is shown in table I. The prevalence of NTM in these studies varied from 0.1- 28.1% (table I, figure 2). As shown in the table II, smear microscopy and culture

Figure 1. A study selection process for meta-analysis

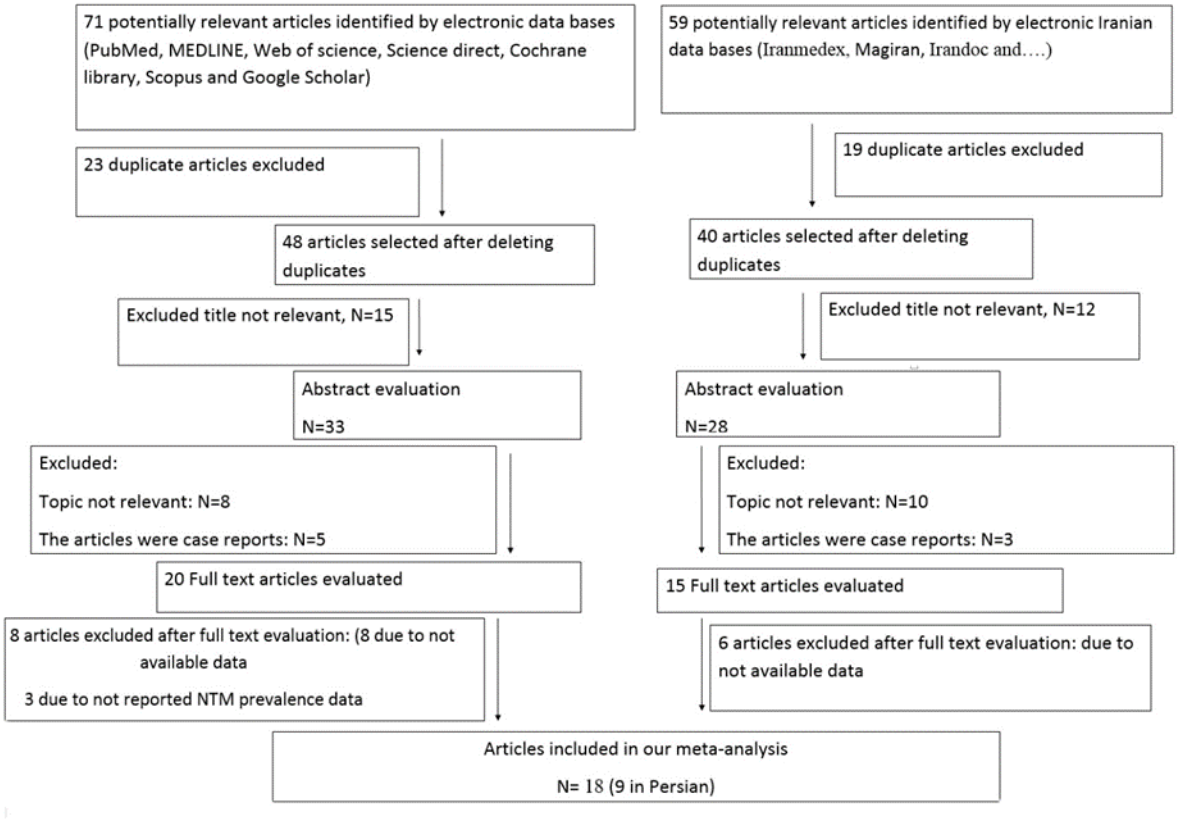

Table 1. Characteristics of selected studies for meta-analysis.

study	Time of study	Publication (years)	Location	Sample size	NTM	Age (years)	Prevalence Of NTM (%)
Mohammadi(11)	1993	1998	Tehran	2272	30	-	1.3%
Bahrmand(12)	1993-1994	1996	Tehran	6472	82	30-69	1.3%
Derakhshani nejad(13)	2004-2011	2014	Tehran	8322	124	57±18.9	1.5%
Nasiri(14)	2010-2012	2014	Tehran	6426	9	11-80	0.1%
Heidari(15)	2007-2008	2009	Tehran	371	43	14-80	11.6%
Moniri(16)	1998-1999	2001	Isfahan	100	6	75.5±16.6	6%
Shafipour(17)	2010-2011	2013	Golestan	3336	16	44±23.3	0.5%
Javid(18)	2007-2008	2009	Golestan	104	17	14- ≤65	16.3
Nasrollahi(19)	2010-2011	2012	Mazandaran	1345	6	45.5±17.93	0.4%
Moghtaderi(20)	2000-2010	2011	E. Azarbaijan	235	15	-	6.4%
Heidar Nejad(21)	2001	2001	E. Azarbaijan	165	10	44.01±18.23	6.1%
Naserpour-Farivar(22)	2002-2004	2006	Sistan-Baluchestan	210	59	20-≤60	28.1%
Naderi(23)	2004	2006	Sistan-Baluchestan	150	20	50≤-≤50	13.3%
Namaei(24)	2002	2003	R.Khorasan	1700	8	-	0.5%
Hashemi- Shahraki(25)	2008-2012	2014	Khuzestan	2313	92	-	4%
Hashemi Shahraki(26)	2009-2012	2013	khuzestan	190	23	48.3-57.1	12.1%
Roayaei(27)	1993-1994	1996	Khuzestan	6031	18	-	0.3%
Khosravi(28)	2007-2008	2009	Khuzestan	150	8	24-36	5.3%

Table 1. Detection and identification process for non-tuberculous mycobacteria (NTM) in different studies

Location	Province	First author	Detection	Identification
Center	Tehran	Bahrmand	Smear microscopy and Culture on Lowenstein Jensen	Biochemical tests, pigment production and growth rate
	Tehran	Derakhshani nejad	Smear microscopy and Culture on Lowenstein Jensen	Biochemical tests, pigment production and growth rate, PCR RFLP of hsp65
	Tehran	Heidari	Smear microscopy and Culture on Lowenstein Jensen	Biochemical tests, pigment production and growth rate, Amplification of IS6110, PCR-RFLP for hsp65
	Tehran	Mohammadi	Smear microscopy and Culture on Lowenstein Jensen	Biochemical tests, pigment production and growth rate
	Tehran	Nasiri	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate,
	Isfahan	Moniri	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate
North	Golestan	Javid	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate, PCR for IS6110
	Golestan	Shafipour	Smear microscopy and Culture on Lowenstein Jensen	growth characteristics and pigmentation, biochemical properties, *16S rRNA PCR*
	Mazandaran	Nasrollahi	Smear microscopy and Culture on Lowenstein Jensen	Biochemical tests, pigment production and growth rate, RFLP PCR and Resay enzyme
West	E. Azarbaijan	Heidar nejad	Smear microscopy and Culture on Lowenstein Jensen	Growth characteristics and pigmentation, biochemical properties,
	E. Azarbaijan	Moghtaderi	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate
East	Sistan-baluchestan	Naserpour-Farivar	Smear microscopy and Culture on Lowenstein Jensen	Growth characteristics and pigmentation, biochemical properties
	Sistan-baluchestan	Naderi	Smear microscopy and Culture on Lowenstein Jensen	Growth characteristics and pigmentation, biochemical properties
	R. Khorasan	Namaei	Smear microscopy and Culture on Lowenstein Jensen	Growth characteristics and pigmentation, biochemical properties
South	Khuzestan	Hashemi -Shahrki	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate, Amplification and sequencing of *16S rRNA* , *rpoB*, *hsp65*, and *IT S* ,mesa
	Khuzestan	Hashemi Shahraki	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate, Amplification and sequencing of *16S rRNA* , *rpoB*, *hsp65*, and *IT S*
	khuzestan	Khosravi	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate, PCR RFLP of hsp65
	khuzestan	Roayaei	Smear microscopy and Culture on Lowenstein Jensen	Morphology, biochemical tests, pigment production and growth rate

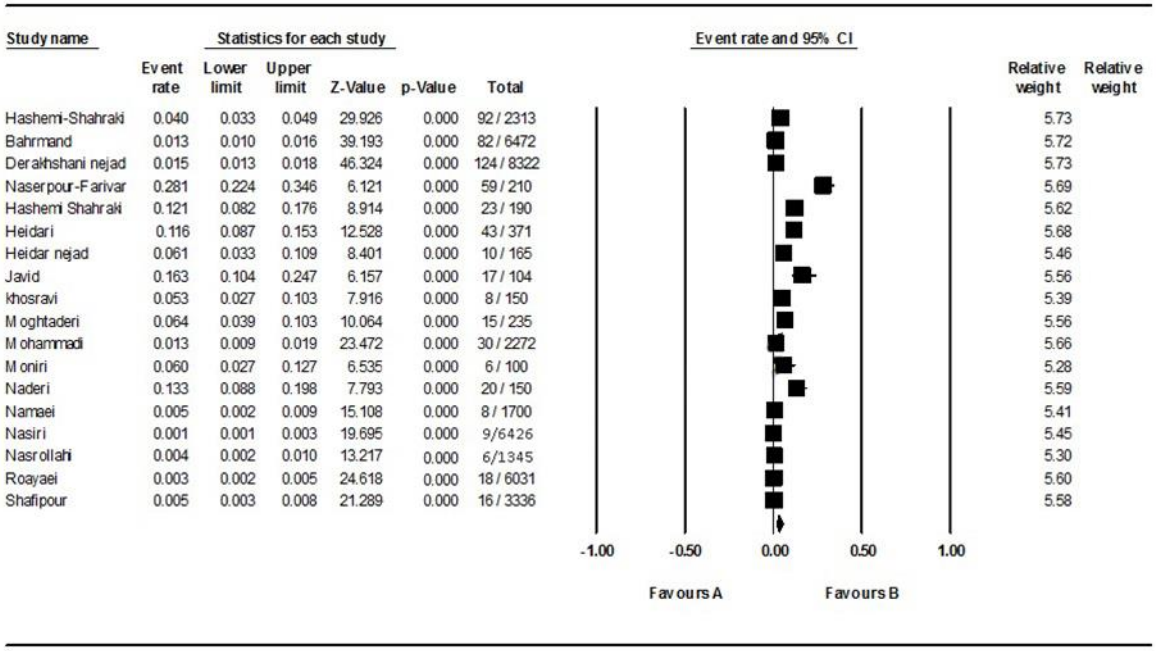

Figure 2. Forest plot of the meta-analysis on prevalence of NTM in Iranian clinical specimens.

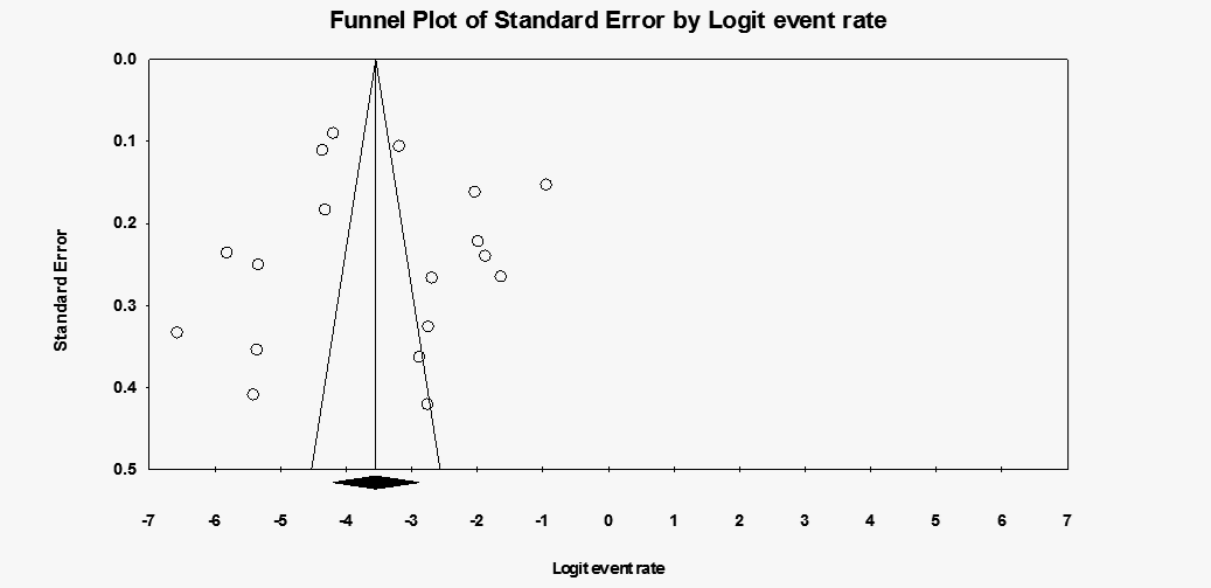

Figure 3. Funnel plot of the meta-analysis on prevalence of NTM in Iranian clinical specimens.

Table 3. Sub-groups meta-analysis of NTM prevalence in Iranian clinical specimens.

Subgroups	No. of studies	Random model				Test of heterogeneity	
		NTM prevalence (95% Cl)[%]	Z	Value of *p*	Q	Value of *p*	I^2 (%)
Overall effects	18	1.3 (1.1, 1.5)	10.61	< 0.001	952.34	> 0.05	98.2
Research year ≥ 2004	10	1. 6 (1.1,2.1)	77.60	< 0.001	452.9	> 0.05	98
Research year < 2004	6	0. 9 (0.7,2.9)	11.08	< 0.001	84.53	> 0.05	94.08
Sample size ≥ 300	10	1. 1 (1,1.2)	13.3	< 0.001	396.5	> 0.05	97.7
Sample size ≤ 300	8	1 2.2 (10.5,14.1)	23.32	< 0.001	65.7	> 0.05	89.3
North	3	0. 8 (0.6,1.1)	3.093	< 0.001	119.48	> 0.05	98.3
South	4	1.6 (0.7,11.7)	48.33	< 0.001	150.6	> 0.05	98
East	3	4.2 (1.8,3.7)	28.49	0.015	131.3	> 0.05	98.4
West	2	6. 3 (4.3,9.1)	13.11	0.08	0.017	-	-
Center	6	1. 2 (1.1,1.4)	74.30	< 0.001	223.9	> 0.05	98

Z= Z Value, Q= Cochrane test, CI= Confidence Interval

on Lowenstein Jensen media were the primarily methods for detection of NTM and identification which were performed by morphology, biochemical tests, pigment production and growth rate in all studies. In addition molecular methods were used in only 27.7% (5/18) of studies.

Overall effects

From the total of 18 separate articles which were included in our meta-analysis, according to heterogeneity tests, there were heterogeneities between studies (Q2 = 952.34, I2 = 98.2, P < 0.001). The random effect model was used for combine the prevalence of NTM. Overall Prevalence of NTM was 1.3% (1.1- 1.5%) (Table III). For evaluation of publications biased, we used from funnel plot (figure 3). For NTM prevalence, the distribution of studies was asymmetrical, suggesting that publications biased may have been presented in our meta-analysis, but Egger weighted regression analysis did not established publication biased (p › 0.05) in our meta-analysis.

Subgroups analysis for NTM prevalence in Iranian clinical specimens

Subgroups analysis showed that the combined prevalence of NTM was higher in studies with sample size ≤ 300 in compared to studies with sample size ≥ 300 (12.2%, 95% Cl (10.5- 14.1%)) vs. (1.1%, 95% Cl (1- 1.2%)). The analysis based on time of study indicated that prevalence was higher in studies which were performed after 2004 in compared to studies which were performed before 2004 (1.6%, 95% Cal (1.4-1.7%) vs. (0. 9%, 95% Cl (0.8-1.9%). Also this meta-analysis showed that the rate of NTM is varied in different geographical parts and the rate of NTM was higher in the West of Iran (6. 3%, 95% Cl (4.3-9.1%) in comparison with the other geographical parts in Iran (table III).

Subgroups analysis for distribution of different species of NTM

Table IV presents the subgroups analysis for distribution of different species of NTM. The most prevalent slow growing mycobacteria was *M. simiae* (44.2%, 95% Cl (37.3- 51.2%)) and the most prevalent rapid growing mycobacteria was *Mycobacterium fortuitum* (14.3%, 95% Cl (11.2-

18%). Test of heterogeneity for *M. simiae* was (p = 11.63, I2 = 82.81) in comparison to heterogeneity test for *M. fortuitum* (P = 25.04, I2 = 72.4). In our study, *M. terrae* and *M. avium-intracellular* were the second and third most frequently reported NTM species in the clinical specimens, with prevalence (18.3%, 95% Cl (11.3- 28.2%) and (16.5%, 95%Cl (6-37.9%),(table IV). Distribution and prevalence of NTM in clinical specimens from various areas of Iran is different, this diversity has shown in figure 4.

Assessment of sensitivity analysis

We assessed the sensitivity analysis by removal of the study that had the biggest sample size (13) and the study that had the smallest sample size (16) or the study with highest prevalence of NTM (13), the assessment indicated that the meta-analysis estimates not changed.

Discussion

Until now, there is no detailed meta-analysis about NTM prevalence in Iran and few studies have addressed this issue (29).

Overall, this meta-analysis indicated that prevalence of NTM in Iranian clinical specimens was 1.3% (1.1-1.5%). Based on the subgroups analysis was observed that the prevalence of NTM was higher when sample size was ≤ 300, the reasons for this is not completely known, but it can be assumed that in such studies more selected samples were included. It is also possible that the smaller sample size have a higher rate of random errors compared to the larger sample size (30). According to the time of study, the present study revealed that prevalence was higher in studies were performed after 2004 in compared to those were performed before 2004. The reasons for this, could be; active investigating for NTM, improvement in detection techniques, present of more susceptible hosts that increased NTM infections in community (31), the increase in prevalence of chronic lung diseases, and as well as likelihood of a change in the ecology of NTM (32).

In regard to the geographic areas, most of studies were conducted in central part of Iran (Tehran), but NTM prevalence was not high in Tehran. The prevalence rate of NTM in Tehran reflecting the prevalence rate of NTM in Iranian clinical specimens, because Tehran as the capital of Iran have many health care centers and play referral role for all areas of Iran and patients with suspected tuberculosis from around of Iran are referred to the Tehran for better management and further follow up (14). The prevalence of NTM in the west and east of country was high, the reasons for this may be due to the neighborhood of these area to countries (Afghanistan, Pakistan, Iraq and turkey) with high load of some NTM species such as *M. fortuitum* and *M. chelonae* (33, 34).

In general, as mentioned in results, according to our meta-analysis, *M. simiae* was the most prevalent NTM in Iranian clinical specimens. The prevalence of *M. simiae* in different studies was variable, with frequency of 1.5 - 10% (35). *M. simiae* is slow growing mycobacteria, and like the *M. tuberculosis* is the only NTM that its niacin test positive, which may manifested with clinical and radiological signs consistent with tuberculosis. Unfortunately, *M. simiae* is almost resistant against first-line anti-TB treatment, so anti-TB medication will lead to treatment failure. Unlike most other NTM species, this species was frequently seen in HIV-negative patients (36). Earlier, *M. terrae* described as non-pathogenic mycobacteria, but studies have proved that this species could be responsible for some clinical syndromes (31, 37). In United State, Asia and many parts of Europe, the most prevalent NTM is MAC complex (38). Also in many studies MAC complex is the most predominant NTM among nontuberculous mycobacteria-related pulmonary infections, especially in HIV-positive patients (39). Its prevalence in pulmonary NTM disease reported as 43-81% (40). Similar to our study, the highest prevalence among rapid growing

mycobacteria in other parts of the world was associated to *M. fortuitum* (31, 41-43).

Strengths and weaknesses of the review

This review has several strengths. We performed a comprehensive search for articles by searching multiple data bases and also papers selection was done independently by two reviewers. Disagreements between reviewers were resolved with discussion. Meta-analysis was performed in accordance with published guidelines and for reduction of heterogeneity, we performed subgroups analysis. This review has some limitations. We didn't contact the authors of the studies to obtain additional information in cases that needed clarification, so meta-analysis was performed based on available information in our selected articles. Also we are not aware of studies that have been conducted but unpublished yet, so they are not included in our study.

Conclusion

This meta-analysis review shows prevalence and distribution of NTM in Iranian clinical specimens. Our results showed that *M. simiae* is the most prevalent NTM among Iranian clinical specimens, and this species of NTM may be seen with clinical and radiological manifestations consistent with tuberculosis, Unfortunately, *M. simiae* is almost resistant against first-line anti-TB treatment, so anti-TB medication will lead to treatment failure. Therefore, there is an urgent need for application of new diagnostic techniques for identification and effective drug susceptibility tests for NTM species in Iran, and more attention should be paid to the NTM and their infections.

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Conflict of interest

None declared

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