Prevalence of nontuberculous mycobacteria isolated from environmental samples in Iran: A meta-analysis

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INTRODUCTION

Environmental opportunistic mycobacteria are found in natural and human and can cause disease in humans and animals. The atypical and nontuberculous mycobacteria (NTMs) names are used for these organisms.[1] In relation to Runyon’s classification, based on growth rates and pigment production, NTMs are classified to Groups I to IV in which Groups I to III are slow-growing NTMs and Group IV are rapid-growing NTMs.[2] While most NTM species are considered opportunistic pathogens, but some of them are related powerfully with several human diseases, for example, pulmonary infection, nosocomial infections, hypersensitivity, pneumonitis, asthma, gang ionic infection, and infection of skin/soft tissue.[3] Infections are present in immunocompetent and immunocompromised humans, more often HIV-positive patients all over the world.[4‑6] Because of increasing rates of NTMs infections, there is interest to identify the source of the NTMs transmission.[7] The possible ways for infect the human beings are: Ingestion or inhalation and may have a 2-fold effect.[8]

NTMs are isolated from natural waters, water distribution systems, drinking waters, soil, food, biofilms, aerosols, and dust.[9] A study conducted in 1999[10] showed that the ideal condition for more growth of NTM species was

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Background: While the most nontuberculous mycobacteria (NTMs) species are considered as opportunistic pathogens, some of them are related to several human infections. It is believed that environment is the main source for these infections. Distribution and scattering pattern of NTMs has not been well studied in Iran and a few studies about this subject have been done, so the aim of this study was to determine prevalence of NTMs in environmental samples from Iran. Materials and Methods: Data about prevalence of NTMs in environmental samples from Iran were obtained by searching databases. The studies presenting cross-sectional or cohort and the papers with sample size ≥30 were included. Then, the meta-analysis was performed using Comprehensive Meta-Analysis software and Cochran’s Q and P tests. The strategy search was based PRISMA protocol is available online (PRISMA, http://www.prisma-statement.org). Results: The results of this meta-analysis showed that overall combined prevalence of NTMs in environmental samples from Iran was 38.3%. The frequency of NTM was higher in the north of Iran (73.2%). The most prevalent rapid-growing mycobacterium was Mycobacterium fortuitum (19.8%), and the most dominant slow-growing mycobacterium was Mycobacterium flavescens (16.8%). Conclusion: In regard to increasing incidence of disease in immunocompromised patients and existence of different types of mycobacteria species in environmental samples, efforts should be focused on measures that will specifically remove NTMs from habitats where susceptible individuals are exposed.

Key words: Meta-analysis, nontuberculous mycobacteria, prevalence

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aquatic environments, low pH values with the presence of high organic matter concentrations, with presence of low numbers of heterotrophic bacteria, in versus Bland et al.\textsuperscript{[10]} presented that the ideal conditions were presence of large numbers of heterotrophic bacteria, low temperature, alkaline characteristic, and high conductivity of water.

NTM species are very resistant to conventional disinfection techniques, particularly chlorination.\textsuperscript{[11]} The most important NTM studied is Mycobacterium avium because can grow in conditions with temperatures from 0 to 50°C and pH values between 3 and 8.\textsuperscript{[12]} Geographic distribution in prevalence of NTM species is different. For example, Mycobacterium malmoense is most prevalent in Europe than the USA. A few species of rapidly growing mycobacteria such as Mycobacterium abscessus, Mycobacterium chelonei, and Mycobacterium fortuitum are opportunistic pathogens and not saprophytes and can produce various diseases in human. Like their slowly growing relatives, members of these species are very resistant to antibiotics and conventional disinfectants and they are normal residents of water and animal reservoirs.\textsuperscript{[9]} Due to the increasing immunocompromised diseases in the world and high rate of NTMs in various geographical regions, understanding the NTMs species distribution and prevalence in environment is necessary. Distribution and scattering pattern of NTM has not been well studied in Iran and a few studies about this subject have been done,\textsuperscript{[13]} so the aim of this meta-analysis was to determine prevalence of NTMs in environmental samples from Iran. The results of our meta-analysis can give comprehensive data about distribution, prevalence, and diversity of NTMs in environmental samples.

MATERIALS AND METHODS

Search strategies
The searching process (PRISMA, \url{http://www.prisma-statement.org}) was conducted for prevalence of environmental-NTMs in Iran from papers that were published by the end of 2014 using Web of Science, PubMed, Scopus, MEDLINE, Cochrane Library, ScienceDirect, Google Scholar, and the Scientific Information Database. The original articles published in English and Persian were included in our research. The keywords such as environmental nontuberculous mycobacteria, atypical mycobacteria, NTM infection or NTM diseases, distribution, prevalence, incidence, and Iran were used for searching process. Likewise, the searching was done with similar strategies and related Persian keywords among Iranian databases. We searched Scientific Information Database (www.sid.ir), Iranmedex (www.irannmedex.com), magiran (www.Magiran.com), and Irandoc (www.irandoc.ac.ir). Furthermore, we checked references from retrieved papers in both English and Persian for additional data. Two reviewers independently searched the databases with the similar way and they reviewed the titles, abstracts, and full texts to determine if they met eligibility criteria for inclusion. To identify further papers, references in these papers were reviewed. The last search was done by the end of May 2015.

Criteria for inclusion and exclusion
The studies presenting cross-sectional or cohort on the prevalence of environmental-NTMs were included in our meta-analysis. The papers with sample size ≥30 which report the prevalence of NTMs in environmental samples by the end of 2014 were selected. We excluded review articles, congress and meeting abstracts, papers reported in languages other than English or Persian, meta-analysis or systematic reviews, abstract forms of papers, case report papers, duplicate publication of the same paper. To reduce the risk of errors, two reviewers independently completed this process.

Extraction of data
A data abstraction form for reviewers was designed. The following data were included in these forms: The first author’s name, time of the study, year of publication, location of study, and the prevalence of NTMs.

Statistical analysis
Analysis of data was performed by Comprehensive Meta-Analysis Software Version 3.3.070 (Bio stat Company). Prevalence was reported by 95% confidence intervals (CIs). Random effects 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 and I² tests. To evaluate possible publication bias, Egger weighted regression method was used ($P < 0.05$ was considered as indicative of a statistically significant publication bias). For check of publication bias, the funnel plot was used; further, for likely asymmetrical distribution of studies, the Egger’s linear regression test was applied. Subgroups analysis was performed for NTMs prevalence and distribution of different species of NTMs in environmental samples. Finally, sensitivity meta-analysis was assessed.

RESULTS

Characteristics of selected studies
The study inclusion process is shown in Figure 1. In brief, initially using multiple databases, 83 relevant papers were recognized. Forty papers were excluded because of duplication, and due to the irrelevant titles, 19 papers were excluded. Then, 15 full texts papers were evaluated, nine papers were excluded (due to topic of four papers were irrelevant, four papers were case report, and one were review article). Eight papers were deleted after full-text evaluation because they did not report NTM...
prevalence. Finally, seven papers were included in this meta-analysis. The features of included papers in this meta-analysis are summarized in Table 1. Majority of studies were directed in Center of Iran [Table 1]; among total seven studies from three geographical locations of Iran, five cases (71.4%) were reported from Center of Iran (two cases from Tehran province and three from Isfahan province), from both West (West Azarbaijan) and North (Golestan) provinces was reported one case (14.2%). In included studies, the prevalence of NTMs varied from 18.6% to 73.2% [Table 1 and Figure 2]. The primary detection carried out based on phenotypic tests such as smear microscopy and culture on Lowenstein–Jensen medium, and identification was done by morphology, biochemical tests, pigment production, and growth rate. From total studies, 2 (28.5%) of those have used from molecular methods and the other studies have used only from phenotypic tests for identification.

Overall effects
From the total papers selected for our meta-analysis, in accordance with heterogeneity test, there were heterogeneities between studies (Q² = 173.623, I² = 96.54, t = 23, P < 0.001); therefore, we used random model to combine the prevalence of NTMs in environmental samples. In regarding overall meta-analysis, the combined prevalence of NTMs was 38.3% (95% CI [35.7, 40.9%]) [Table 2]. Funnel plot was used for check of publication bias [Figure 3]. In regarding likely asymmetrical distribution of studies, the Egger’s linear regression test was used; the result of this test did not confirm the publication bias in our meta-analysis (P > 0.05).

Subgroups analysis for nontuberculous mycobacteria prevalence in environmental samples from Iran
Subgroups analysis presented that the prevalence of NTMs in environmental samples was higher in studies with sample size ≥ 100 in contrast with studies with sample size ≤ 100 ([43.4%, 95% CI (18.2, 72.5%)] vs. [29%, 95% CI (21.4, 34.5%)]). This meta-analysis also showed that the frequency of NTMs in environmental samples was varied in different geographical areas, and the frequency of NTM was higher in the north of Iran (73.2%, 95% CI [66.9, 78.6%]) in comparison with the other geographical areas in Iran [Table 2 and Figure 3].

Subgroups analysis for distribution of different species of nontuberculous mycobacteria in environmental samples
Table 3 outlines the subgroups analysis for distribution of different species of NTMs in environmental samples. The most prevalent rapid-growing mycobacterium was M. fortuitum (19.8%, 95% CI [16.6, 23.6%]) and the most dominant slow-growing mycobacterium was Mycobacterium flavescens (16.8%, 95% CI [12.8, 21.8%]). The frequency of M. fortuitum as highest prevalent NTM species in environmental samples from all geographical locations of Iran is shown in Table 3. The heterogeneity test for M. fortuitum was (P ≤ 0.001, F = 35) in contrast with

Table 1: Characteristics of included studies for meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Time of study</th>
<th>Publication</th>
<th>Location</th>
<th>Sample size</th>
<th>Number of NTMs</th>
<th>Prevalence of NTM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falsafi[14]</td>
<td>-</td>
<td>2014</td>
<td>Tehran</td>
<td>38</td>
<td>21</td>
<td>55.3</td>
</tr>
<tr>
<td>Rahideh et al.[15]</td>
<td>2012</td>
<td>2013</td>
<td>Tehran</td>
<td>454</td>
<td>193</td>
<td>42.5</td>
</tr>
<tr>
<td>Nasr-Esfahani et al.[16]</td>
<td>-</td>
<td>2012</td>
<td>Esfahan</td>
<td>85</td>
<td>22</td>
<td>25.9</td>
</tr>
<tr>
<td>Rahbar et al.[20]</td>
<td>2004</td>
<td>2010</td>
<td>West Azarbaijan</td>
<td>350</td>
<td>65</td>
<td>18.6</td>
</tr>
</tbody>
</table>

NTMs = Nontuberculous mycobacteria
heterogeneity test for \( M. \) flavescens (\( P = 0.152, I^2 = 43.2 \)). In this study, the second and third highest frequencies of NTM species in the environmental samples were \( M. \) chelonae and \( Mycobacterium \) simiae, with prevalence ([14.5%, 95% CI (10.9, 19%)] and [14.3%, 95% CI (4.7, 36.1%)]) [Table 3].

### Assessment for sensitivity analysis

By exclusion of the studies that had the biggest\(^{[15]}\) or smallest sample size\(^{[14]}\) or the study with highest rate of NTMs,\(^{[15]}\) the sensitivity of meta-analysis was assessed; the assessment revealed that the meta-analysis estimates was not changed.

### DISCUSSION

This study is an extensive meta-analysis on prevalence of NTMs in environmental samples from Iran. The result of our study provides secondary (synthesized) epidemiological information on prevalence of NTMs based on results of research studies in different regions of a climatically heterogeneous of our country. Furthermore, it prepares the baseline information to design a more effective strategy for the national control programs. This study focused on published papers by the end of 2014 to guess a more exact estimation of prevalence of NTMs in environmental samples. Over the recent decades, the ratio of isolation of tubercle bacilli has dropped in the developed countries whereas the occurrence of infection with NTMs has risen.\(^{[21]}\)

In general, this meta-analysis showed that prevalence of NTMs in environmental samples from Iran was 38.3% (35.7, 40.9%), but this result cannot reflect the real prevalence of environmental-NTMs in different regions of Iran because these data are from several provinces. The number of studies about NTMs in Iran is few, for that cannot generalize these results to whole our country. The reasons for no attention to the NTMs are: Transmission of NTM between people is said to be really rare and NTM disease was not reported and treatment is not obligatory.\(^{[22]}\) However, the high prevalence of NTMs in our country may be due to the neighborhood of Iran with countries such as Afghanistan, Pakistan, and Iraq with high load of NTMs.\(^{[23]}\) This high prevalence of NTM in soil and water samples can have subsequent consequences; for example, it can affect the efficacy of BCG vaccine and prior sensitivization with environmental mycobacteria inhibit induction of BCG-mediated immune response and reduce the protection against \( Mycobacterium tuberculosis \)\(^{[24,25]}\) and presence of environmental mycobacteria in a region cause the immune system of humans have contact with these NTMs and then in immunocompromised humans cause...


Table 3: Subgroups meta-analysis for rapidly growing mycobacteria and slow-growing mycobacteria species distribution among selected studies

<table>
<thead>
<tr>
<th>Subgroups RGM</th>
<th>Number of study</th>
<th>Random model</th>
<th>Heterogeneity test</th>
<th>Egger's test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NTM prevalence (95% CI) (%)</td>
<td>Z</td>
<td>P</td>
</tr>
<tr>
<td>Mycobacterium fortuitum</td>
<td>7</td>
<td>19.8 (16.6-23.6)</td>
<td>10.6 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium chelonae</td>
<td>5</td>
<td>14.5 (10.9-19)</td>
<td>10.2 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium smegmatis</td>
<td>3</td>
<td>7.5 (3.8-14.2)</td>
<td>4.4 &lt;0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Mycobacterium fallax</td>
<td>3</td>
<td>3 (1.3-6.4)</td>
<td>8.3 &lt;0.001</td>
<td>0.748</td>
</tr>
<tr>
<td>Mycobacterium phlei</td>
<td>3</td>
<td>7.9 (4.9-12.5)</td>
<td>9.3 &lt;0.001</td>
<td>0.709</td>
</tr>
<tr>
<td>Mycobacterium auropense</td>
<td>1</td>
<td>9.5 (2.4-31.1)</td>
<td>3.02 0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium poriferae</td>
<td>1</td>
<td>5.2 (3.3-8.2)</td>
<td>5.2 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium obuense</td>
<td>1</td>
<td>5.2 (3.3-8.2)</td>
<td>5.2 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium abscessus</td>
<td>2</td>
<td>1.6 (0.6-4.1)</td>
<td>3.6 &lt;0.001</td>
<td>0.055</td>
</tr>
<tr>
<td>Mycobacterium senegalenses</td>
<td>1</td>
<td>4.7 (2.4-8.7)</td>
<td>8.8 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium peregrinum</td>
<td>2</td>
<td>5.4 (3.2-9)</td>
<td>2.2 0.027</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium aurum</td>
<td>1</td>
<td>0.5 (0.2-0.9)</td>
<td>5.2 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium mucogenicum</td>
<td>6</td>
<td>5.2 (3.3-8.2)</td>
<td>6.06 &lt;0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Mycobacterium chiake</td>
<td>1</td>
<td>4.5 (0.6-26.1)</td>
<td>2.9 0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium duvalii</td>
<td>1</td>
<td>4.5 (0.6-26.1)</td>
<td>2.9 0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium neoaureum</td>
<td>2</td>
<td>3.4 (1.1-10.2)</td>
<td>5.6 &lt;0.001</td>
<td>0.746</td>
</tr>
<tr>
<td>Mycobacterium simiae</td>
<td>1</td>
<td>14.3 (4.7-36.1)</td>
<td>2.8 0.004</td>
<td>-</td>
</tr>
<tr>
<td>Mycobacterium terrae</td>
<td>3</td>
<td>5.9 (3.4-10.1)</td>
<td>9.2 &lt;0.001</td>
<td>0.54</td>
</tr>
<tr>
<td>Mycobacterium triviale</td>
<td>1</td>
<td>6.2 (3.4-11.2)</td>
<td>8.3 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium kansasi</td>
<td>2</td>
<td>3.3 (1.5-7.1)</td>
<td>1.5 0.122</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAC</td>
<td>2</td>
<td>4.8 (1.2-17.1)</td>
<td>4.1 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium gordonae</td>
<td>5</td>
<td>4.1 (2.2-7.4)</td>
<td>6 &lt;0.001</td>
<td>0.181</td>
</tr>
<tr>
<td>Mycobacterium gastri</td>
<td>1</td>
<td>1.2 (0.3-4.8)</td>
<td>6.1 &lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mycobacterium xenopi</td>
<td>2</td>
<td>4.8 (1.2-17.1)</td>
<td>4.1 &lt;0.001</td>
<td>0.27</td>
</tr>
<tr>
<td>Mycobacterium flavescent</td>
<td>4</td>
<td>16.8 (12.8-21.8)</td>
<td>5.7 &lt;0.001</td>
<td>0.152</td>
</tr>
<tr>
<td>Mycobacterium thermoresistible</td>
<td>2</td>
<td>10.6 (7.2-15.4)</td>
<td>6.1 &lt;0.001</td>
<td>0.175</td>
</tr>
</tbody>
</table>

RGM = Rapidly growing mycobacteria; CI = Confidence interval; NTM = Nontuberculous mycobacteria; MAC = Mycobacterium avium complex

![Funnel plot for meta-analysis on prevalence of nontuberculous mycobacteria in environmental samples from Iran](image)

Figure 3: Funnel plot for meta-analysis on prevalence of nontuberculous mycobacteria in environmental samples from Iran

Possibly produce cutaneous or subcutaneous infections, such as furunculosis which is caused by *M. fortuitum* in immunocompromised persons.[23] According to subgroups analysis, we noticed that the combined prevalence of NTMs was higher when sample size was ≥100; the reasons for this are unknown, and probably due to the larger sample size, the normal distribution was seen in the prevalence. The prevalence of NTMs in environmental samples from the north (Golestan province) was higher than other regions of Iran; the reasons for this may be because neighborhood of this region with countries around the Caspian Sea that have similar patterns in prevalence of NTMs and likely both of those have similarity in climate condition and composition of plant flora.[26] Prevalence of NTMs in environmental samples in various regions of Iran was varied (18.6–73.2%); this is probably due to the difference in geographical locations, climate condition, and use of different decontamination processes.[23] Previous studies have shown that the most of NTMs isolated from soil are rapid growers though some slow-growing species have been accounted;[26] the meta-analysis showed the similar pattern as the most NTMs isolated from environmental samples were rapid-growing species, and
the most common NTM species among rapid-growing mycobacteria were *M. fortuitum*, followed by *M. chelonae* and the highest frequent NTM species among slow-growing mycobacteria were belonged to the *M. flavescens* and *M. simiae*. *M. flavescens*, was considered as a nonpathogenic bacterium to humans. Its recovery has constantly been connected to either contamination in the laboratory, but there are some case reports about *M. flavescens* which can cause pneumonia related to constitutional symptoms, disseminated infections, and glottal abscess.\(^{[31]}\) Numerous evidences suggest that there is a direct relationship between the prevalence of environmental mycobacteria isolated from clinical samples in an area with an abundance of NTMs in the environment,\(^{[39]}\) as well as the clinical manifestations of NTMs are often mixed with infections result from *M. tuberculosis* that is a problematic subject in diagnosis of NTM; the NTMs can cause false positive in detection by direct sputum smear microscopy.\(^{[32]}\) Different studies conducted on clinical samples from Iran showed that the most commonly isolated NTMs are *M. fortuitum* and *M. simiae\(^{[33‑35]}\) which were comparable with our meta-analysis and confirmed that probably the source of clinical isolates was from environment and person-to-person transmission was not proved.\(^{[36]}\) For quick and accurate identification of NTM species directly from clinical samples, molecular methods are essential tools in clinical microbiology and medical laboratories.\(^{[37]}\) However, unfortunately, the lack of standard molecular techniques to detect NTMs with high sensitivity, overlaps of infections caused by NTMs with tuberculosis, the lack of facilities for access to environmental samples, and lack of cooperation from companies and organizations for obtaining samples have encountered current and future researches in this field. Contamination is a critical issue in health-care centers, and NTMs monitoring assessment should be evaluated by infection prevention and control professionals.\(^{[3]}\) Public and environmental efforts should have focused on measures that will specifically remove NTMs from habitats where susceptible individuals are exposed.\(^{[38]}\) NTMs distribution in Environment is characterized by existence of many immunocompromised individuals in communities and high prevalence of NTMs in environment which can help clinicians to take proper treatment measures and strategies. This study has some strength; we accomplished a comprehensive study for articles by search in multiple databases and also articles selecting was completed independently by two investigators. In some cases, differences among investigators were resolved with debate. Meta-analysis was performed in consistent with available published guidelines and for decreasing the heterogeneity, subgroups analysis was done. However, there are some limitations. We did not contact the authors of the Original selected studies to achieve further information in cases which needed to elucidation, so meta-analysis was performed only based on available information in the selected papers as well as we were not aware of studies that have been carried out, but still unpublished, so they were not included in present study.

### CONCLUSION

In regard to increasing incidence of disease in immunocompromised patients and existence of different types of mycobacteria species in environmental samples, efforts should be focused on measures that will specifically remove NTMs from habitats where susceptible individuals are exposed.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

### AUTHORS’ CONTRIBUTIONS

This work was conducted in collaboration between all authors. AKh, AB, and DE designed the study and wrote the protocol, the first draft of the manuscript, and KGh revised the final draft of the manuscript. DM and AKh contributed to search and collect data. AT performed the statistical analysis.

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

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