

1 **Prevalence of Nontuberculous Mycobacteria and the Emergence of Rare**
2 **Species in Henan Province, China**

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28 **Abstract**

29

30 Nontuberculous mycobacteria (NTM) is a large group other than the *Mycobacterium*
31 *tuberculosis* complex and *Mycobacterium leprae*. Epidemiological investigations have
32 found that the incidence of NTM infection is increasing in China, and it is naturally
33 resistant to many antibiotics. Therefore, studies of NTM species in clinical isolates are
34 useful for understanding the epidemiology. The current study aimed to investigate the
35 incidence and types of NTM species. Of the 420 samples, 285 were positive for
36 *Mycobacterium tuberculosis*, 67 samples were negative, and the remaining 73 samples
37 contained NTM; including 35 (8.3%) only NTM, and 38 (9%) mixed (*M. tuberculosis*
38 and NTM). The most prevalent NTM species were *M. intracellulare* (30.1%), followed
39 by *M. abscessus* (15%) and *M. triviale* (12%). *M. gordonae* infection was detected in
40 9.5% of total NTM-positive cases. Moreover, the study reports the presence of *M.*
41 *nonchromogenicum* infection and a high prevalence of *M. triviale* for the first time in
42 Henan. *M. intracellulare* is the most prevalent, accompanied by some emerging NTM
43 species, including *M. nonchromogenicum* and a high prevalence of *M. triviale* in the
44 Henan region. Monitoring NTM transmission and epidemiology could enhance
45 mycobacteriosis management in the future.

46 **Keywords:** Mycobacteria; Infectious disease epidemiology; Nontuberculous
47 mycobacteria.

48 **Introduction**

49 Nontuberculous mycobacteria (NTM) is a large group other than the *Mycobacterium*
50 tuberculosis complex and *Mycobacterium leprae*. In recent years, the isolation rate of
51 NTM has been increasing annually, attracting the attention of healthcare management
52 and researchers [1]. So far, over 200 species and 19 subspecies of NTM have been
53 identified [2]. Recent data showed an upward trajectory in pulmonary NTM infection
54 (82.1%) and disease (66.7%). The overall annual rate of change for NTM infection and
55 disease per 100,000 persons/year was 4.0% and 4.1%, respectively. The absolute
56 numbers of NTM infection and disease showed an overall annual change of 2.0 and 0.5,
57 respectively. *Mycobacterium avium* complex infection and disease and *Mycobacterium*
58 abscessus complex infection displayed increasing trends [3]

59 Like many other countries, China faces the emerging challenges of NTM infections due
60 to the increasing prevalence of conditions such as chronic obstructive pulmonary
61 disease (COPD), the growing population of elderly people, and immunocompromised
62 patients. These conditions may contribute to a higher risk of NTM infections.
63 Additionally, advancements in diagnostic techniques, including Fujifilm SILVAMP
64 (FujiLAM; Japan) for TB LAM test to rapidly detect *M. tuberculosis* [22] and increased
65 awareness among healthcare professionals might lead to improved detection and
66 reporting of NTM infections, including *M. triviale* infections.

67 According to a nationwide multicenter study published in 2021, *M. intracellulare*
68 (52.6%) had the highest isolation rate among NTM in China, followed by the
69 *Mycobacterium abscessus* complex (23.1%) [4]. In Guangzhou, China, the isolation
70 rate of NTM is higher than the national average, with *Mycobacterium avium*-
71 *intracellulare* complex (44.5%), *Mycobacterium abscessus* complex (40.5%), and
72 *Mycobacterium kansasii* (10.0%) being the most prevalent [5].

73 The emergence of NTM infections may further complicate tuberculosis (TB) treatment
74 and diagnosis in the near future [3]. Recently (Data not published), many NTM have
75 also been observed in health centers. However, species and subspecies identification
76 data are unavailable in some regions, including the Nanyang. Pulmonary NTM
77 infections primarily manifest as chronic lung diseases that resemble TB, with symptoms

78 like cough, fatigue, weight loss, and difficulty breathing [6]. Extrapulmonary NTM
79 infections can affect various body parts, including skin, lymph nodes, joints, and bones,
80 causing a wide spectrum of symptoms depending on the site of infection [7].

81 The diagnosis of NTM infections is mainly based on clinical, radiological, and
82 microbiological results [8]. The clinical course of NTM pulmonary infection is
83 heterogeneous, and the disease is associated with considerable mortality and morbidity
84 [9]. Treatment decisions for NTM infections depend on identifying isolated species,
85 drug sensitivity testing, and disease severity [9]. However, identifying NTM species is
86 not performed in some regions, which may lead to its transmission to the population.
87 The NTM are abundantly found in the soil and water, which may transmit from animals
88 to humans [10]. In Thailand and Vietnam, NTM constitutes 21% of mycobacterial
89 infection, and those who develop lung disease are at high risk of developing NTM
90 infections. Out of 218 patients, 30% were potential NTM pulmonary disease, 4% were
91 confirmed, and 5% were diagnosed with disseminated NTM disease. Patients with HIV
92 infection and primary immunodeficiency may develop NTM pulmonary disease as part
93 of disseminated infections [11].

94 Diagnosing NTM infections can be challenging, as the symptoms are nonspecific and
95 overlap with other respiratory conditions [12]. Definitive diagnosis involves isolating
96 the NTM bacteria from clinical samples and identifying the specific species through
97 laboratory techniques.

98 Among the pathogenic NTM species, *M. triviale* is rare in humans (0.09% of detected
99 NTMs) [13]. *M. avium* and *M. intracellulare* are most common (80% of all NTM)
100 [14,15]. Much information has been accumulated for the major organisms, including *M.*
101 *avium*, *M. intracellulare*, *M. kansasii*, *M. abscessus*, and *M. fortuitum*. However, little is
102 known about the other rare NTMs, including *M. triviale*.

103 The Mycobacterium abscessus complex (MABC) is a rapidly growing NTM that can
104 cause human diseases. The MABC is widely distributed in various environments and
105 consists of three subspecies: *Mycobacterium abscessus subsp. abscessus* (Mab),
106 *Mycobacterium abscessus subsp. massiliense* (Mma), and *Mycobacterium abscessus*
107 *subsp. bolletii* (Mbo) [16].

108 Although the distribution of MABC is regional, it is the main NTM causing respiratory
109 infections worldwide, accounting for up to 80% [17]. It most commonly occurs in
110 immunocompromised patients, such as those with cystic fibrosis (C.F.), HIV-positive
111 patients, COPD patients, and bronchiectasis patients [17]. MABC-induced pulmonary
112 infections have become an important global threat to C.F. patients. They accelerate
113 inflammatory lung damage, leading to increased morbidity and mortality [18].

114 Due to the diverse nature of NTM species, their varying antibiotic susceptibilities, and
115 the complexity of infections [19], managing NTM infections requires a
116 multidisciplinary approach involving infectious disease specialists, pulmonologists,
117 and other relevant healthcare professionals [20]. Patients with suspected NTM
118 infections must seek medical attention, undergo proper diagnostic testing, and adhere
119 to the prescribed treatment regimen under healthcare professionals' guidance.

120 In summary, understanding the distribution of NTM is crucial for clinical treatment
121 decisions. Furthermore, exploring the correlation between phenotypic drug
122 susceptibility and genetic mutations has profound implications for the rapid prediction
123 of drug susceptibility. In essence, research on NTM is critical for addressing the
124 challenges posed by these infections, improving patient outcomes, developing effective
125 public health strategies, and advancing our understanding of the complex interactions
126 between these bacteria and human health. The current study was designed to understand
127 the distribution of NTM in clinically isolated samples in Henan Chest Hospital.

128 **2. Methodology:**

129 **2.1 Ethical approval number**

130 This study was ethically approved by the Ethics Committee of Henan Chest Hospital,
131 China (Henan Chest Hospital-2023). Informed consent has been taken from each
132 patient. However, data has not been linked to individual patients.

133 **2.2 Samples collection**

134 A total of 420 TB suspect sputum samples were collected from patients referred to the
135 Henan Chest Hospital Laboratory. The suspects sought a diagnosis at Henan Province,
136 China's Tuberculosis Clinical Research Centre. Medical records and routine analysis,

137 including species identification, patient demographic characteristics, gender, age,
138 regimens, and previous history and outcomes.

139 **2.3 Sample processing and culturing**

140 All the samples were cultured on MGIT and Lowenstein-Jensen (LJ). Single colonies
141 were picked from LJ. Culture medium (Baso Diagnostics Inc., Zhuhai, China),
142 inoculated into 7H9 supplemented with Tween-80 (0.05%) and OADC (10%) and
143 placed at 37 °C on a shaking incubator.

144 **2.4 Identification**

145 All NTM isolates were identified using the Kangli Medical Service Mycobacterial
146 Species Identification Array Kit (TBC and NTM nucleic acid mass spectrometry
147 detection (rapid detection + strain identification)
148 (<http://www.kanglijianyan.com/Product/Detail/471>) following the manufacturer's
149 guidelines and conduct appropriate quality control measures to ensure reliable results.
150 The Kit identifies 8 *M. tuberculosis* complex (MTBC) and more than 32 NTM species
151 (including *M. chelonae*, *M. kansasii*, *M. intracellulare*, etc. with only one test.

152 It utilizes nucleic acid mass spectrometry, allowing for rapid detection, strain and
153 identification. By employing this single test, clinicians and researchers can accurately
154 identify the presence of MTBC and a wide range of NTM responsible for various
155 human infections.

156 **3. Results**

157 **3.1 Prevalence of NTM species**

158
159 A total of 420 samples under suspicion were subjected to screening for NTM and *Mtb*
160 infections. Out of these, 188 (44.7%) samples were females, and 232 (55.3%) were
161 males, resulting in a gender distribution of 1:1.2, and the mean age of patients was 41.2
162 years. Among the 420 samples, 358 (85.2%) were positive, including 285 (67.8%)
163 positive for *Mtb* infection and 73 (17.3%) positive for NTM. Among the NTM
164 infections, 35 (8.3%) were pure infections, and 38 (9%) were mixed infections
165 containing both *Mtb* and NTM. 62 (14.7%) samples were negative, as detailed in
166 Supplementary File S1.

167

168 In the current study, *M. intracellulare* was the most prevalent NTM species (Table 2),
169 followed by *Mycobacterium abscessus* (*M. abscessus*), *M. chelonae*, and *M. triviale*. *M.*

170 *abscessus* was detected in eleven isolates (15%, 11/73), including four mixed samples
171 (*M. abscessus* and Mtb), two were mixed with *M. intracellulare* (*M. intracellulare* / *M.*
172 *abscessus*), and five were only infected with *M. abscessus*.

173

174 *M. intracellulare* was the most prevalent species in Henan Chest Hospital (n=23/73,
175 30.1%) (Table 2). This high prevalence of *M. intracellulare* among pure NTM and
176 mixed infection is alarming. Eleven patients were solely infected with *M. intracellulare*
177 (11/73, 15%), and one had *M. branderi*/*M. intracellulare*, and one with *M. chelonae* /
178 *M. intracellulare*. Eight isolates were mixed (8/73, 10.9%) and infected with Mtb/ *M.*
179 *intracellulare* and one Mtb/*M. intracellulare*/*M. chelonae*. One mixed infection of *M.*
180 *branderi*/*M. intracellulare* has also been reported for the first time in our study (Table
181 2).

182 Despite its name, *M. triviale* has garnered interest in medical and scientific
183 communities due to its potential implications for human health. *M. triviale* rarely found
184 TB infection. However, nine isolates (9/73, 12.3%) were *M. triviale*. Three were mixed
185 infections with Mtb, and the remaining six were purely *M. triviale* (Table 2). The rate
186 of *M. triviale* in pure NTM infected and mixed infection was high in the current study
187 (12.3%).

188

189 *M. chelonae* was detected in ten samples (13%, 10/73), including *M. chelonae* / *M.*
190 *intracellulare*. Most of these were mixed, including eight mixed infections with Mtb;
191 one was Mtb, *M. intracellulare*, and *M. chelonae*. The current study found only one
192 sample, purely *M. chelonae* (Table 2).

193 The other low-frequency NTM infection has been given in Table 3. Of 73 NTM-
194 positive cases, seven (9.5%) samples were infected with *M. gordonae* with Mtb,
195 including one solely infected with *M. gordonae* (Table 3), three isolates were *M. flavum*
196 (4.1%), one was *M. triplex* and *M. Kansasii* each. All these were mixed infections,
197 including Mtb and NTM species. The other rare NTM species have been given in
198 supplementary file S1.

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204 **Discussion**

205 Studies on NTM are essential for better public health management as they provide a
206 better understanding of epidemiology to develop effective strategies for preventing,
207 diagnosing, and treating NTM infections, ultimately improving communities' overall
208 health outcomes. Usually, species identification of NTM is not performed in clinical
209 practice, so misdiagnosis and incorrect treatment are commonly initiated, which may
210 lead to more severe conditions in the future and cause a significant economic burden
211 on healthcare systems and individuals.

212 In the current study, we analyzed about 18 months (July 2021 to March 2023) to find
213 the prevalence of NTM species in Henan. To our knowledge, it is the first
214 comprehensive study that reports the prevalence of NTM from Henan province. The
215 distribution of NTM species in China varies across different regions. In Beijing, *M.*
216 *abscessus* complex (MABC) was 73.8% (76/103) and 26.2% (27/103), respectively
217 [21]. Wu et al. reported 5.9% NTM in Shanghai [22] while Gao et al. identified 43
218 (43/200, 21%) as MABC, including 32 (74.4%) *M. abscessus* (Mab) and 11 (25.6%)
219 *Mycobacterium abscessus subsp. massiliense* (Mma) [23]. Similarly, Mab, Mbo, and
220 Mma accounted for 75.2% (97/129), 14.7% (19/129), and 10.1% (13/129) of the MABC
221 isolates, respectively [24]. Compared to this study, there are significant differences in
222 each NTM species infection in clinical settings. Yu et al. reported 4.0% (160/3995)
223 NTM infection in southern-central China [25]. In a recent study, the NTM rate was 6.4%
224 (317/4917) in overall China and 7.7% in southern China [26]. Our study is the first to
225 investigate the distribution of NTM species in Henan, China, thus providing additional
226 data for the southern region. However, pulmonary infections due to NTM are increasing
227 globally [27].

228 The increased prevalence of pulmonary NTM is a major health issue in China [28]. In
229 the current study, the most prevalent NTM was *M. intracellulare*, followed by *M.*
230 *abscessus*. *M. intracellulare* is closely related to *Mycobacterium avium* and is
231 commonly referred to as Mycobacterium avium complex (MAC) along with
232 *Mycobacterium avium* [27]. *M. intracellulare* is an opportunistic pathogen that can
233 cause a group of Mycobacterium avium complex infections. Similar to our results, a

234 previous study [29] from Zhejiang Province reported 6.2% (24/390) NTM in TB-
235 positive cases. The most prevalent NTM species was *M. intracellulare* (16/24, 66.7%),
236 followed by *M. abscessus* (3/24). Zhang et al. analyzed 452 NTM isolates in which *M.*
237 *intracellulare* (188/452; 41.6%) was the most prevalent. Species identification is
238 important for effective treatment because *M. intracellulare* is more sensitive to
239 moxifloxacin and linezolid than *M. avium* species, which are more susceptible to RIF
240 [28].

241 Mixed infection of NTM and Mtb may further complicate the treatment outcomes [30].
242 About 2.6% of infections are mixed infections of NTM and Mtb [30]. The prevalence
243 rates of mixed infections are different in regions, ranging from zero % in Beijing (0/213)
244 to 3.4% (12/353) in Fuzhou and 3.7% in Guangzhou, China [31]. In our study, 38 (9%)
245 isolates were mixed infections. This high mixed infection in our study may further
246 affect the efforts against TB prevention in the future.

247 Mixed infections involving *M. chelonae* and Mtb are rare but may occur in complex
248 with other NTM [32]. *M. chelonae*, a rapidly growing NTM [33], and Mtb, the causative
249 agent of TB, represent distinct species with different clinical characteristics. Diagnostic
250 challenges can arise when these two pathogens coexist in the same host. A successful
251 strategy for handling mixed infections should consider the variations in antimicrobial
252 susceptibility profiles and treatment options for each species involved.

253 Infections caused by *M. triviale* are rare and infrequently reported in clinical practice.
254 *M. triviale* rarely causes respiratory disease in humans and accounts for only 0.09% of
255 NTM species [34]. However, contrary to the previous reports, the current study found
256 that *M. triviale* was Henan's third most prevalent NTM (Table 3). *M. triviale* is generally
257 considered non-pathogenic [34]. However, there have been isolated cases where *M.*
258 *triviale* has been associated with opportunistic infections in individuals with
259 compromised immune systems or pre-existing lung conditions [34]. These infections,
260 although uncommon, underscore the importance of understanding the potential
261 interactions between environmental mycobacteria and human health. Due to its
262 minimal virulence and infrequent association with TB disease, *M. triviale* infections are

263 not as extensively studied. Investigating epidemiology and transmission dynamics can
264 provide insights into effective management strategies and prevalence among diverse
265 populations. Looking into the high prevalence rate of this rare pathogen, molecular
266 characterization and treatment methods for this bacterium are still to be elucidated for
267 better management of NTM in China.

268 A single mixed infection of *M. triplex* and Mtb was also detected (Table 5). This
269 pathogen is slow-growing and rarely causes human disease. *M. flavum* has been known
270 to occasionally cause infections in humans, particularly in individuals with
271 compromised immune systems or underlying lung conditions. Some other rare NTM
272 species detected in the current study include *M. gordonae* and *M. nonchromogenicum*
273 (Table 5). Previous studies report *M. gordonae* is causing disease in immunosuppressed
274 and immunocompetent patients [35,36]. Tsukamura *et al.*, for the first time, reported *M.*
275 *nonchromogenicum* as a pathogenic NTM, causing pulmonary infections [37]. Later,
276 some other studies also reported the pathogenic nature of *M. nonchromogenicum*
277 [8,38,39]. However, infections of *M. nonchromogenicum* are very rare and have not
278 been reported in China.

279 Here, we report the first case of *M. nonchromogenicum* in Henan, China, and *M.*
280 *branderi*. These NTM have been sporadically isolated from cases involving pulmonary
281 and wound lesions observed in individuals with intact immune systems [40,41].
282 However, our case stands out as a unique instance, as it marks the first documented
283 occurrence of this species in an individual with compromised immunity, specifically a
284 patient diagnosed with Acute Myeloid Leukemia (AML). This observation suggests a
285 broader range of potential infections for *M. branderi* than previously recognized,
286 encompassing patients with weakened immune systems.

287 Studies on NTM clinical issues are limited, including the diverse range of NTM species,
288 limited and heterogeneous clinical data, absence of standardized diagnostic criteria,
289 small sample sizes, geographic variations, selection and publication biases,
290 retrospective designs leading to recall bias, inconsistent antibiotic susceptibility testing
291 methods, incomplete understanding of pathogenicity, difficulties in assessing long-term
292 outcomes and interactions with host factors. Despite these challenges, these studies are

293 vital for enhancing our comprehension of NTM infections and refining patient
294 management, necessitating careful consideration of these limitations in research design
295 and analysis to ensure robust and applicable outcomes.

296 **Limitations of the current study**

297 The current study has some limitations. Firstly, the dataset's size is limited, and patient
298 sociodemographic data remains absent. Additionally, the exploration of the
299 transmission network was not within the scope of this research.

300 **Recommendations**

301 Future research on NTM should focus on expanding data collection with larger sample sizes and
302 incorporating patient sociodemographic information to understand infection dynamics better.
303 Additionally, epidemiological studies investigating drug resistance patterns among NTM species
304 and developing evidence-based treatment guidelines, especially for mixed infections involving Mtb
305 and NTM, may improve patient management. Longitudinal studies tracking infection progression,
306 exploring geographical variations in prevalence, and advanced genomic analysis, along with One
307 health approaches, may provide insights into NTM evolution, transmission, and sources.

308 **4. Conclusion:**

309 In the current study, most NTM infections are mixed with Mtb. The *M. intracellulare*
310 was the most prevalent NTM, followed by *M. abscessus*, *M. chelonae*, and *M. triviale*
311 in Henan Chest Hospital. We report for the first time a high prevalence of respiratory
312 infection caused by a very rare pathogen, *M. triviale*. Some other rare NTM, including
313 *M. gordonae*, *M. nonchromogenicum*, and *M. triplex*, were also detected. NTM
314 infections are not limited to specific regions and can have global health implications.
315 Understanding the distribution and transmission dynamics of NTM worldwide is
316 crucial for coordinated efforts in surveillance, prevention, and control on an
317 international scale. Mixed infections involving NTM and Mtb represent a complex and
318 intriguing intersection of mycobacterial pathogens. Clinicians must navigate the
319 complexities of balancing treatment protocols and monitoring patients closely to ensure
320 successful outcomes, making multidisciplinary collaboration and individualized care

321 paramount in addressing this unique challenge at the intersection of tuberculosis and
322 NTM infections.

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325 **Consent for publication**

326 Not applicable as we did not link the individual results with a specific person.

327 **Conflict of interest**

328 All authors have no conflicts of interest.

329 **Supplementary Materials:** Supplementary File S1

330 **Author Contributions:** "Conceptualization, L.R. and L.J.; methodology, S.H, D.C.Z.;
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341

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451 **Table 1. Summary of 420 TB patients**

No	Infection type	Number
1.	TB	285
2.	TB+NTM	38
3.	NTM	35
4.	Negative	62

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454 **Table 2. Prevalence of *M. abscessus* and its mixed infections**

No.	Infection/Samples	NTM specie	Frequency
1.	Mtb and NTM	<i>M. abscessus</i>	4
2.	NTM	<i>M. abscessus</i>	5
3.	NTM	<i>M. abscessus/ M. intracellulare</i>	2
4.	NTM	<i>M. intracellulare</i>	11
5.	Mtb and NTM	<i>M. intracellulare</i>	7
6.	NTM	<i>M. intracellulare/M. branderi</i>	1
7.	Mtb and NTM	<i>M. intracellulare/M. chelonae /</i>	1
8.	Mtb and NTM	<i>M. triviale</i>	3
9.	NTM	<i>M. triviale</i>	6
10.	Mtb and NTM	<i>M. chelonae</i>	8
11.	NTM	<i>M. chelonae</i>	1
12.	Mtb and NTM	<i>M. chelonae / M. intracellulare</i>	1

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457 **Table 3. Other NTM infections with tuberculosis**

No.	Infection	NTM specie	Frequency
1.	Mtb and NTM	<i>M. gordonae</i>	6
2.	Mtb and NTM	<i>M. nonchromogenicum</i>	1
3.	Mtb and NTM	<i>M. triplex</i>	1
4.	Mtb and NTM	<i>M. kansasii</i>	1
5.	NTM only	<i>M. flavum</i>	2
6.	NTM only	<i>M. myxogenes</i>	1
7.	Mtb and NTM	<i>M. flavum</i>	1
8.	NTM only	<i>M. gordonae</i>	1

458 Mtb: *Mycobacterium tuberculosis*

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