Microbes and Infectious Diseases 2024; 5(1): 155-167

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Nontuberculous mycobacterial infection in patients with bronchiectasis. A single-center study

Marwa Elmongi [1], Mohammed F. Elkenawy [1], Tamer A. Elhadidy [2], Rasha M. Elnagar [1,3]*

1- Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
2- Chest medicine department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
3- Department of Basic Medical Sciences, College of Medicine, AlMaarefa University, Riyadh, Saudi Arabia.

ARTICLE INFO

Article history:
Received 10 October 2023
Received in revised form 4 Novamber 2023
Accepted 7 Novamber 2023

Keywords:
Atypical mycobacteria
NTM
Bronchiectasis

ABSTRACT

Background: Globally, bronchiectasis-related nontuberculous mycobacterial (NTM) disease is on the rise. The diagnosis of NTM infection is difficult and treatment varies according to NTM species. This study intended to detect the prevalence of NTM in patients with bronchiectasis. **Methods:** A total of 100 bronchoalveolar lavage (BAL) specimens were collected from patients with adult-onset bronchiectasis attending the chest medicine department, Mansoura University Hospitals (MUH), Egypt between December 2021 and November 2022. Clinical samples were processed to detect NTM isolates by phenotypic methods including microscopic examination, culture on Lowenstein Jensen (LJ) slants, and various biochemical reactions. Positive NTM specimens were further confirmed by multiplex polymerase chain reaction (PCR). **Results:** Out of 100 patients included in this study, seven cases were positive for NTM in their BAL by conventional methods. Of those, two were confirmed as Mycobacterium kanssasi by multiplex PCR (29%; 2/7) and five (71.4%, 5/7) were unidentified NTM species. NTM infection was significantly associated with old age and immunocompromised status of the patients. **Conclusion:** Nontuberculous mycobacterial (NTM) lung disease is a common health problem in bronchiectasis and should be routinely examined particularly in old age and individuals with lower immune response.

Introduction

Nontuberculous mycobacteria (NTM), commonly referred to as opportunistic, environmental, or atypical mycobacteria, are pathogens other than Mycobacterium leprae and Mycobacterium tuberculosis complex. They have been isolated from soil, water, and shower aerosols, which are thought to be the natural sources of NTM infection [1]. Occasionally, NTM can infect humans or animals, causing a variety of pulmonary and extra-pulmonary infections [2]. Even though more than 180 species have been identified to date, only a few of them have been linked to human illness, including rapidly growing NTM as *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum*; and slow-growing NTM as *Mycobacterium avium* complex (MAC), *Mycobacterium kansasii (M. kansasii)*, *Mycobacterium xenopi*, *Mycobacterium simiae*, *Mycobacterium malmoense* and *Mycobacterium szulgai* [3].

DOI: 10.21608/MID.2023.241803.1633
* *Corresponding* author: Rasha M. Elnagar
E-mail address: drrasha_m@mans.edu.eg

Both immunocompetent and immunocompromised individuals are affected by the increased prevalence of NTM lung disease [4]. Being common environmental organisms, genetic predisposition, immunological deficiencies, anatomic lung diseases, and humidity are among the risk factors that increase the rate of pulmonary NTM infection [5].

Bronchiectasis is a chronic lung disease associated with inflammation, structural lung damage, and decreased mucociliary clearance that is complicated by chronic airway infection, including NTM infection. Furthermore, some studies revealed that NTM might play a direct role in the anatomic change of the bronchi, defective airway clearance, and development of bronchiectasis. Regular screening of NTM in frequently exposed patients should be done to distinguish between active NTM infection and colonization [6].

Bronchiectasis can present either as a localized obstructive disease of a lung lobe or segment or as a diffuse disorder affecting the majority of the lungs. Diffuse bronchiectasis is more likely to be associated with numerous risk factors including cystic fibrosis, immunodeficiency, Aspergillus infection, and NTM infection. Clinical traits, radiographic findings, and microbiological testing all are required for the diagnosis of NTM [7].

Clinical presentation of NTM lung infection is similar to bronchiectasis including, excess sputum production, cough, dyspnea, and hemoptysis [8]. Pneumonic changes, multiple small nodules and cavities, tree-in-bud (TIB) abnormalities, and mosaic perfusion are the main radiographic findings favoring a diagnosis of NTM infection in bronchiectasis. Smear microscopy is a low-cost method for quickly identifying mycobacteria, but it is unable to distinguish between NTM and Mycobacteria tuberculosis [9]. A definitive diagnosis of NTM lung disease relies on the detection of mycobacterial growth in culture media, even though this procedure takes several weeks to produce results. Molecular methods based on nucleic acid amplification offer a potential more rapid and sensitive method for detection of NTM directly from respiratory specimens without the need for culture [10].

The non-specific symptoms, lack of routine screening and few numbers of studies that examined NTM in depth make underestimation of the isolation rate of NTM in bronchiectasis, which may be higher than expected. In the current study, the prevalence of NTM infection in a well-defined group of individuals with adult-onset bronchiectasis will be investigated, and the clinical correlation will be also assessed.

Subjects and methods

Study design

One hundred adult patients with confirmed bronchiectasis were enrolled in this prospective cohort study during the period from December 2021 to November 2022. Patient were recruited from chest medicine department, Mansoura University Hospital (MUH), Egypt. Patients diagnosed as mycobacterial tuberculosis were excluded. Each participant was subjected to history taking including demographic data (age, sex, and residence), underlying medical conditions, and smoking habit.

Samples collection and processing

Following the recommendations of the American Thoracic Society (ATS), bronchoscopy was used to obtain bronchoalveolar lavage (BAL) samples [11]. The specimens were delivered right away to the Microbiology Diagnostics and Infection Control Unit (MDICU), Medical Microbiology and Immunology Department, Mansoura college of Medicine, Egypt. In the event of a delay, samples were stored at 4 °C.

After samples were centrifuged for 15 minutes at 3000 rpm, sediments were utilized for both Ziehl-Neelsen staining (Egyptian Diagnostic Media (EDM), and culture on Lowenstein Jensen (LJ) media slants (Accumix, India). All culture plates were incubated aerobically at 37°C and inspected weekly for 4-6 weeks. Dry, rough, raised, irregular colonies of NTM were identified [2].

For pigment production cultures were exposed to light for 1h and then re-incubated again for 24-48h; NTM cultures that produced yellow to orange pigment during exposure to light only and no pigment production in the dark were considered photochromogens; while those that produced yellow, orange or red colonies during incubation in the dark were named scotochromogens. Cultures that produce no pigments either during incubation in the dark or light were considered non-photochromogens [12].

The isolated NTM were distinguished using biochemical reactions such as urea hydrolysis tests, catalase tests, and nitrate reduction tests purchased from Mast Group Ltd., Merseyside, UK; growth in

the presence of 5% NaCl in LJ medium (FIPCO, Egypt), and growth on MacConkey's agar without crystal violet (Liofilchem, Italy) [13].

Molecular identification of NTM by multiplex polymerase chain reaction (PCR)

Colonies of NTM on LJ medium slants were verified by multiplex PCR based on identification of the 16S rRNA, and internal transcribed spacer (ITS) using the following primers obtained from (Enzynomics, South Korea) **(Table 1).** NTM colonies were suspended in 1.0 ml of the TE buffer, boiled for 15 minutes at 95°C, and then frozen for 30 min at -20°C. The mixture was centrifuged to create pellet and then kept at -80°C to facilitate further DNA amplification [14].

The thermal cycler conditions used for DNA amplification were initial denaturation at 95°C for 15 min, 30 cycles of denaturation at 95°C for 1min, primer annealing at 62°C for 1 min, extension at 72°C for 60 sec, and finally elongation at 72°C for 10 min. After operating at 100 V for 1 hour, amplicons were seen on a 1.5 % agarose gel containing Ethidium bromide (Sigma, USA). The 100 bp DNA marker (Promega, USA) was used to estimate the length of the PCR products [15].

Ethical approval

The Ethical Committee of the Faculty of Medicine, Mansoura University examined and approved this work (R.21.06.1549). All of the participants in this study gave written informed consent.

Data analysis

The Statistical Package of Social Science (SPSS) program version 24 (SPSS Inc., Chicago, IL, USA) was used. The qualitative data were described in terms of numbers and percentages. The mean ± SD (standard deviation) was used to represent continuous variables. The Chi-square test, Fisher exact test, and independent t-test were used to compare distinct variables. When $p \leq 0.05$, the results were deemed significant.

Results

One hundred clinically diagnosed adult bronchiectasis patients were included in this study. Their age ranges from 32 to 77 years with a mean age of 52.11± 10.99. The study results showed a male preponderance with 54 (54.0%) patients, while 46 (46.0%) were females. About 52% of the studied patients were from urban residences whilst 48% were from rural areas. Sixty-six patients (66.0%) were non-smokers, 22% were active smokers and 12% were ex-smokers. Among the studied patients, 55% of the patients were immunocompromised including diabetes mellitus (DM), rheumatoid arthritis, systemic lupus erythematosus, and cancer, whereas 72.0% were on prolonged antibiotic therapy **(Table 2).**

The overall prevalence of NTM amongst studied patients was 7% (n= 7/100) by phenotypic methods. DNA amplification of isolated NTM using multiplex PCR showed that *Mycobacterium kanssasi* represented (28.6%, 2/7), while other species of isolated NTM represented (71.4%, 5/7) as described in **figures (1, 2)** and **table (3).**

Patients with positive NTM disease had higher mean age (60.00±7.59) in comparison to negative NTM patients (51.51±11.0) **(Figure 3)**; furthermore, there was a significant correlation between NTM infection and older age (*P* value 0.048). In addition, the NTM infection was significantly correlated with the immune status of the patients (*P* value 0.016) as all NTM positive cases suffered from weak immunity; 3 cases had DM, 3 cases had solid organ malignancy and 1 patient had bronchogenic carcinoma. However, there was no discernible difference in terms of gender, place of residence, smoking, body mass index (BMI), or prolonged antibiotic therapy between positive NTM infection and negative cases (*P* values were 0.700, 0.707, 0.479, 0.885, and 0.402 respectively) **(Table 4).**

Table 1. Primers used in the study and amplicon size.

Primer	Sequence (5'-3')	Target NTM	Amplicon size (bp)	Reference
16S rRNA	TGAGATACGGCCCAGACTCCT CTCTAGACGCGTCCTGTGCAT	Pan-mycobacterial species	688	15
ITS	CAACAGCAAATGATTGCCAG CACATTTCGATGAACGCCG	*Mycobacterium avium* complex	169	
ITS	ATCCCAACAAGTGGGGTGC CGCTACCCGTAGGGCAACG	*Mycobacterium kansasii*	218	
16S rRNA	CCTTTCTAAGGAGCACCATTT CGAGCGAGGCTATGTTTAGAT	*Mycobacterium abscessus*	271	

Table 2. Demographic data and characteristics of the 100 studied patients.

111	**n (%)**
Age (Years)	
Mean ± SD	52.11± 10.99
Min-Max	32-77
Age categories	
< 40 y	21 (21.0%)
40-60 y	54 (54.0%)
>60 y	25 (25.0%)
Sex	
Male	54 (54.0%)
Female	46 (46.0%)
Residence	
Urban	52 (52.0%)
Rural	48 (48.0%)
Smoking	
Non smoker	66 (66.0%)
Smoker	22 (22.0%)
Ex- smoker	12 (12.0%)
BMI*	
Underweight	31 (31.0%)
Normal	39 (39.0%)
Obese	30 (30.0%)
Immune status	
Immunocompromised	55 (55.0%)
Immunocompetent	45 (45.0%)
Long term antibiotics	
Yes	72 (72.0%)
No	28 (28.0%)

*BMI: Body mass index

Table 3. Culture, biochemical reactions, and PCR results of the isolated NTM species (n= 7).

Isolated NTM species	**Culture results**	**Pigment production**	**Biochemical reactions**				**PCR results**
			Urease test	**Catalase test**	**Growth in the presence of 5% NaCl**	**Growth in MacConkey's agar without crystal violet**	
NTM 1	>7 days	Non-photochromogens	-	+	-	-	Other NTM
NTM 2	>7 days	Non-photochromogens	-	-	-	-	Other NTM
NTM 3	<7 days	Non-photochromogens	-	+	-	-	Other NTM
NTM 4	>7 days	Photochromogens	+	+	-	-	*M.kansasii*
NTM 5	>7 days	Non-photochromogens	+	-	-	-	Other NTM
NTM 6	>7 days	Non-photochromogens	+	+	-	-	Other NTM
NTM 7	>7 days	Photochromogens	+	+	-	-	*M.kansasii*

Table 4. Association between NTM infection and patients' characteristics.

Patients characteristics	Positive NTM cases (n=7)	Negative NTM cases (n=93)	Test of significance	*P* value
Age (Years)				
Mean ± SD	60.00±7.59	51.51±11.0	t=1.99	0.048*
Sex				
Male	3 (42.9%)	51 (54.8%)	FET	0.700
Female	4 (57.1%)	42 (45.2%)		
Residence				
Urban	3 (42.9%)	49 (52.7%)	FET	0.707
Rural	4 (57.1%)	44 (47.3%)		
Smoking				
Non smoker	4 (57.1%)	62 (66.7%)	MC	0.479
Smoker	1 (14.3%)	21 (22.6%)		
Ex- smoker	2 (28.6%)	10 (10.8%)		
BMI				
Underweight	3 (42.9%)	28 (30.1%)	MC	0.885
Normal	2 (28.6%)	37 (39.8%)		
Obese	2 (28.6%)	28 (30.1%)		
Immune status				
Immunocompromised	7 (100%)	48 (51.6%)	FET	0.016*
Immunocompetent	0 (0%)	45 (48.4%)		
Long term antibiotics				
Yes	6 (85.7%)	66 (71.0%)	χ^2 =0.702	0.402
No	1 (14.3%)	27 (29.0%)		

t: Independent t test; FET: Fisher exact test; MC: monte carlo test; χ^2:Chi square test;*significant p≤0.05

Figure 1. Flow chart describes the study

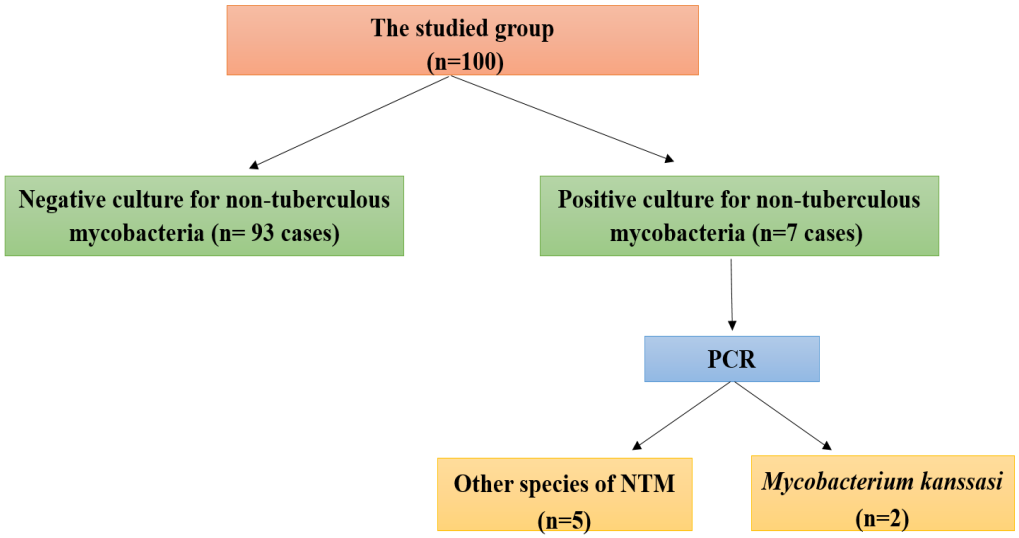


Figure 2. Gel electrophoresis of amplified DNA products of Nontuberculous Mycobacteria (NTM) by multiplex polymerase chain reaction (PCR). Lane 1 M: DNA Ladder (1000 bp); Lanes 4 and 7: positive *Mycobacterium kanssasi* (218bp); Lanes 2, 3, 5, 6, 8-13: negative result for NTM; Lane 14: Negative control.

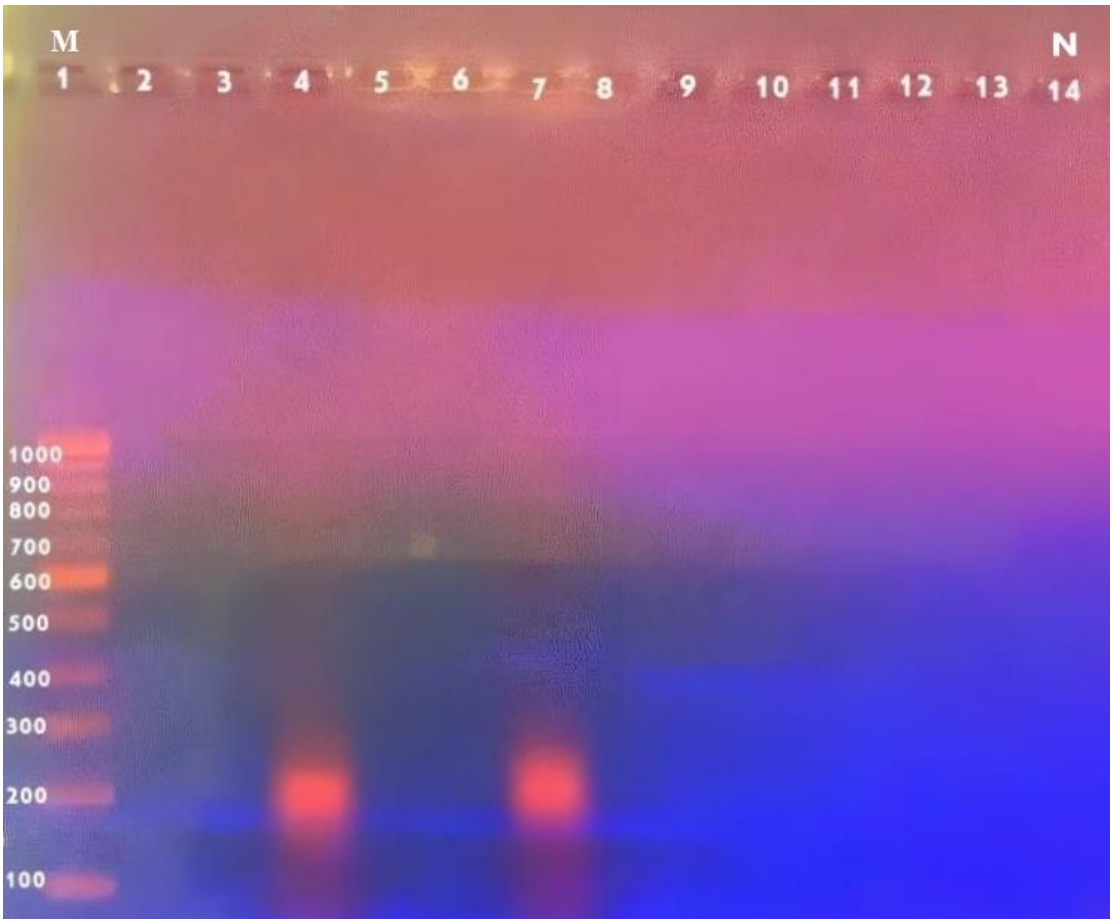
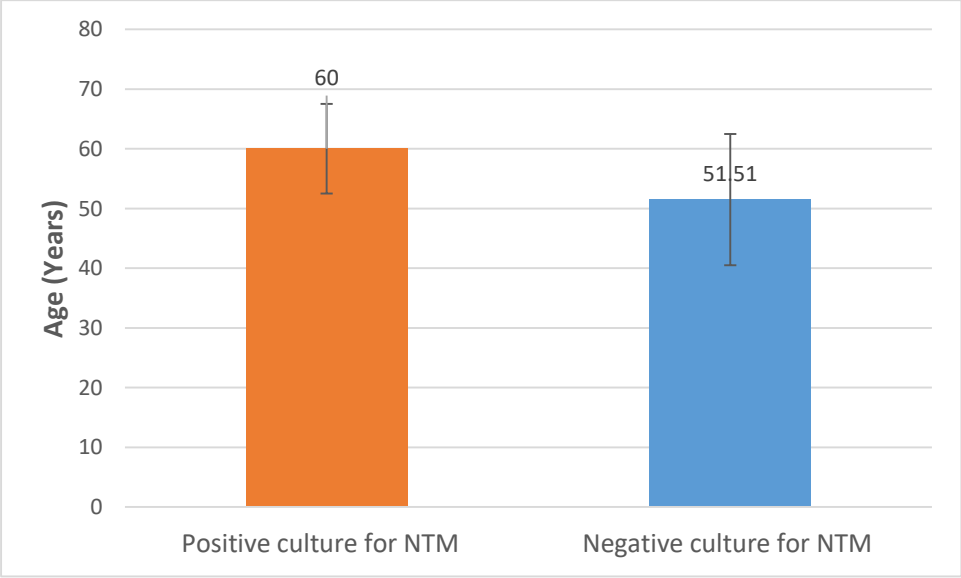


Figure 3. Age distribution between positive and negative NTM cases.



Discussion

Nontuberculous mycobacteria (NTM) are becoming an increasing public health issue that could develop serious infections in both immunocompetent and immunocompromised individuals [16]. Due to their ubiquitous existence in soil and water, humans frequently are exposed. The majority of NTM species are not pathogenic; however, some are able to cause human disease. About 80-90% of all NTM infections appear as pulmonary manifestations [17].

Though normally sterile, the lower respiratory tract in individuals with bronchiectasis can easily become colonized with fungi and NTM, which have a substantial impact on the disease's pathogenesis [18]. It is challenging to make a clinical diagnosis of NTM infection in people with pre-existing lung illness, such as bronchiectasis [19].

About 60% of people with adult-onset bronchiectasis have a history of NTM infection, according to the United States Bronchiectasis Research Registry [20]. Because screening for NTM in respiratory secretions is not routinely required, reporting is not always done, and it can be difficult to distinguish between colonization and active disease, the actual estimates of NTM prevalence in bronchiectasis are unknown. However, a number of variables, such as improvements in diagnostic methods and a growing awareness of NTM infection, have led to an increase in the rates of NTM isolation in recent years [21].

In the present investigation, 100 patients with adult-onset bronchiectasis, including 54 men and 46 women, with a mean age of (52.11± 10.99) years, were assessed for the clinical incidence of NTM infection. The infection rate of NTM infection among the studied patients was 7%. Although this percentage seems low, it was in line with another study conducted in Egypt in which only three NTM were isolated from 40 patients with an infection rate of 7.5% [22]. In addition, our results were similar to another study from Spain where they isolated NTM from sputa of 218 bronchiectasis patients with an infection rate of 8.3 % [23]. In addition, our results were consistent with other researchers from the UK who isolated seven NTM from 80 adult patients with bronchiectasis with a prevalence rate of 8.8 % [24].

In contrast to this study, **Palwatwichai et al.** reported a lower incidence of NTM in bronchiectasis patients (6%) [25]. Additionally, **Pieters et al.** from Netherland, **Sharif et al.** from Pakistan, and **Dhar et al.** from India found a lower prevalence of NTM disease amongst patients with bronchiectasis (5 %, 2.3%, and 0.6%) respectively [26-28]. In addition, several cohort studies from France [29], McDonnell et al., from the UK, **Guan et al. from** China, **King et al.** from Australia, and **Dimakou et al.** from Greece stated low NTM infection rates in bronchiectasis (4 %, 3.9 %, 3.5 %, 2 %, and 0.9 %) respectively [30-33].

Previous studies from the USA, Taiwan, Korea, and Japan found higher incidence rates of NTM pulmonary disease in patients with bronchiectasis, with isolation rates of 50%, 36.5%, 25%, and 17.7%, respectively [34-37]. Similarly, another study from New Zealand described an increased rate of NTM infection with an isolation rate of 10.5% [38]; also, **Faverio et al.** from Italy found a higher rate of NTM infection (12%) [39]. A meta-analysis including 14 studies from multiple geographical regions found that the global prevalence of NTM in adults with bronchiectasis from 2006 to 2021 was 10% [40].

Patients with bronchiectasis are likely predisposed to NTM colonization and subsequent infection because structural abnormalities in the airways impair mucociliary clearance and increase mucus production [41].

There is a difference in the proportions of NTM infection among people with bronchiectasis according to geographical distribution [42]. Based on the American Thoracic Society (ATS) guidelines, 10% to 30% of bronchiectasis patients have NTM lung disease [43]. In a meta-analysis conducted by **Zhu et al.** there was a significant heterogeneity among the included studies, they stated that the highest frequency of NTM in bronchiectasis was in the United States 50%; while lower percentages were described in other regions, such as 9.5% from 8 studies in Asia, 5.4% from 9 studies in Europe, 5.6% from 2 studies in Australia, and 7.5% from 1 study in Africa with overall prevalence of 7.7% [44].

Uncertain ecological factors, such as climate, environmental exposure [45], sample size, collection techniques (sputum versus bronchoalveolar lavage fluid), and detection methods, may all play a role in this geographic heterogeneity [46].

The conventional phenotypic techniques, such as Ziehl-Neelsen (ZN) staining, and various biochemical reactions cannot speciate NTM species; additionally, they are time-consuming and complicated. Molecular techniques may help to differentiate and identify various NTM species from cultures [47]. Herein, multiplex PCR was done to confirm the diagnosis of NTM. Amongst the seven identified NTM isolates by phenotypic methods, 2 (29%) were confirmed as *M. kansasii* by multiplex PCR, while 5 (71%) were non-specific other types of NTM species. In contrast to our findings, a prior study by **Darwish et al.** found that Mycobacterium avium complex (MAC) was the most prevalent NTM (66.6%) and *M. kansasii* represented only (33.4%) [22]. Furthermore, according to **Kwak et al. M. avium**, *M. intracellulare*, and *M. abscessus* were the most frequently isolated NTM from bronchiectasis [48]. Additionally, *M. abscessus* and MAC were shown to be the two most common NTM subspecies in bronchiectasis, according to **Chu et al.** [49]. While in a prior study, MAC was the most common NTM in bronchiectasis [39]. Furthermore, **Wickremasinghe et al.** discovered that in 72% of sputum samples from patients with bronchiectasis, MAC was the main NTM species identified [50].

Another study revealed that *M. simiae* (33%), *Mycobacterium avium*-intracellulare (MAI) (25%), *M. fortuitum* (15%), and *M. abscessus* (10.5%) were the most predominant NTM species in bronchiectasis [21].

The bulk of our NTM-infected patients in this study were over sixty years old. This was in line with findings by **Park et al., Máiz et al.** and **Metersky** and his colleague, and Izumi et al., who discovered that older bronchiectasis patients had a much-increased likelihood of developing NTM disease [18,23,51,52]. This may be owing to impaired pulmonary function and weak immune response in elderly patients.

In this study, there was no correlation between the increased risk of NTM in bronchiectasis and gender, residence, smoking, socioeconomic position, or BMI. This finding was in line with the results of a study by **Zhu et al.** which found that gender, body mass index (BMI), and smoking behaviors were not risk factors for NTM pulmonary illness in bronchiectasis [44]. Our findings, however, were different from those of a prior study that found that individuals with low BMI had a considerably higher incidence of NTM isolation [53]. In addition, contrary to our outcomes, earlier research revealed that females were more likely to have NTM pulmonary disease [19,54].

A previous study demonstrated that women over the age of 50 years are more likely than younger women to have NTM infections; this finding may be related to the menopausal state and a decline in estrogen, which has a protective effect against NTM. In addition, menopause causes a decrease in macrophage colony-stimulating factor serum levels [55].

Studies showed a relationship between the prevalence of NTM lung disorders and a lower socioeconomic level [56]. Unexpectedly, patients with high socioeconomic status who had never smoked before were found to have an elevated risk of NTM development, according to **Shteinberg et al.** [21]. However, these results could be deceiving and overestimated as the increased detection rate in high socio-economic status may be owing to intensive follow-up and increased health consciousness in those patients. In the present study, we did not find any association between the socio-economic status of our patients and the incidence of NTM infection.

Patients with immunocompromised illnesses had a significantly greater rate of NTM infection, however, there was no significant association between increased NTM infection rates in bronchiectasis and prolonged antibiotic use. The increased incidence of NTM in immunocompromised patients was also validated by **Chai et al.** [57].

Conclusion

Adult patients with bronchiectasis are at risk for NTM-pulmonary diseases. Co-infection worsens the disease progression and increases exacerbations. The physicians should be aware of this and include NTM screening in routine bronchiectasis diagnostic workup.

Authors' contributions

Marwa Elmongi was responsible for designing the study protocol, writing the manuscript, and collecting and processing studied samples. Mohammed F. Elkenawy was responsible for supervision of the study. Tamer A. Elhadidy Rasha was responsible for collecting studied samples and clinical data of the patient. Rasha M. Elnagar was responsible for study protocol, data

analysis, writing the manuscript and final revision of the manuscript.

Conflict of interest

None declared.

Funding

None

Availability of data and material

Will be available on reasonable request.

References

1- **Fedrizzi T, Meehan CJ, Grottola A, Giacobazzi E, Fregni Serpini G, Tagliazucchi S, et al.** Genomic characterization of Nontuberculous Mycobacteria. Sci Rep 2017; 7:45258.

2- **Gopalaswamy R, Shanmugam S, Mondal R, Subbian S.** Of tuberculosis and non-tuberculous mycobacterial infections - a comparative analysis of epidemiology, diagnosis and treatment. J Biomed Sci 2020; 27(1):74.

3- **Zweijpfenning SMH, Ingen JV, Hoefsloot W.** Geographic Distribution of Nontuberculous Mycobacteria Isolated from Clinical Specimens: A Systematic Review. Semin Respir Crit Care Med 2018; 39(3):336-342.

4- **Prevots DR, Marras TK.** Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med 2015; 36(1):13-34.

5- **Szymanski EP, Leung JM, Fowler CJ, Haney C, Hsu AP, Chen F, et al.** Pulmonary Nontuberculous Mycobacterial Infection. A Multisystem, Multigenic Disease. Am J Respir Crit Care Med 2015; 192(5):618-28.

6- **Chotirmall SH, McShane PJ.** Time to acknowledge, address, and take action against bronchiectasis. Lancet Glob Health 2019; 7(9):e1162-e1163.

7- **Cowman S, van Ingen J, Griffith DE, Loebinger MR.** Non-tuberculous mycobacterial pulmonary disease. Eur Respir J 2019; 54(1):1900250.

8- **Wagner D, van Ingen J, van der Laan R, Obradovic M.** NTM-NET. Non-tuberculous mycobacterial lung disease in patients with bronchiectasis: perceived risk, severity and guideline adherence in a European physician survey. BMJ Open Respir Res 2020; 7(1):e000498. doi: 10.1136/bmjresp-2019-000498. Erratum in: BMJ Open Respir Res. 2020; 7(1).

9- **Velayati AA, Rahideh S, Nezhad ZD, Farnia P, Mirsaeidi M**. Nontuberculous mycobacteria in Middle East: Current situation and future challenges. Int J Mycobacteriol 2015; 4(1):7-17.

10- **Scoleri GP, Choo JM, Leong LE, Goddard TR, Shephard L, Burr LD, et al.** Culture-Independent Detection of Nontuberculous Mycobacteria in Clinical Respiratory Samples. J Clin Microbiol 2016; 54(9):2395-8.

11- **Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, et al.** American Thoracic Society Committee on BAL in Interstitial Lung Disease. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med 2012; 185(9):1004-14.

12- **Jethva K, Bhatt D, Zaveri M.** Epidemiology of non-tuberculous mycobacteria in India: A review. J pharmacogn phytochem 2019; 8(3): 954-959.

13- **Bhalla GS, Sarao MS, Kalra D, Bandyopadhyay K, John AR.** Methods of phenotypic identification of non-tuberculous mycobacteria. Pract Lab Med 2018; 12:e00107.

14- **Peixoto ADS, Montenegro LML, Lima AS, Melo FL, Barbosa Júnior WL, Neves MMC, et al.** Identification of nontuberculous mycobacteria species by multiplex real-time PCR with high-resolution melting. Rev Soc Bras Med Trop 2020; 53:e20200211.

15- **Singh K, Kumari R, Tripathi R, Gupta S, Anupurba S**. Detection of clinically important non tuberculous mycobacteria (NTM) from pulmonary samples through one-step multiplex PCR assay. BMC Microbiol 2020; 20(1):267.

16- **Zhang ZX, Cherng BPZ, Sng LH, Tan YE.** Clinical and microbiological characteristics of non-tuberculous mycobacteria diseases in Singapore with a focus on pulmonary disease, 2012-2016. BMC Infect Dis 2019; 19(1):436.

17- **Jeon D.** Infection Source and Epidemiology of Nontuberculous Mycobacterial Lung Disease. Tuberc Respir Dis (Seoul) 2019; 82(2):94-101.

18- **Park DI, Kang S, Choi S.** Evaluating the Prevalence and Incidence of Bronchiectasis and Nontuberculous Mycobacteria in South Korea Using the Nationwide Population Data. Int J Environ Res Public Health 2021; 18(17):9029.

19- **Chalmers JD, Crichton M, Goeminne PC, Loebinger MR, Haworth C, Almagro M, et al.** The European Multicentre Bronchiectasis Audit and Research Collaboration (EMBARC): experiences from a successful ERS Clinical Research Collaboration. Breathe (Sheff) 2017; 13(3):180-192.

20- **O'Donnell AE. Bronchiectasis update.** Curr Opin Infect Dis 2018; 31(2):194-198.

21- **Shteinberg M, Stein N, Adir Y, Ken-Dror S, Shitrit D, Bendayan D, et al.** Prevalence, risk factors and prognosis of nontuberculous mycobacterial infection among people with bronchiectasis: a population survey. Eur Respir J 2018; 51(5):1702469.

22- **Darwish AA, El Mahalawy I, El Dahdouh SS, Elkholy RM, El Beshbishy M. I.** Nontuberculous mycobacterium as a hidden cause of noncystic fibrosis bronchiectasis. Egypt J Chest Dis Tuberc 2020; 69(1):46.

23- **Máiz L, Girón R, Olveira C, Vendrell M, Nieto R, Martínez-García MA.** Prevalence and factors associated with nontuberculous mycobacteria in non-cystic fibrosis bronchiectasis: a multicenter observational study. BMC Infect Dis 2016; 16(1):437.

24- **Fowler SJ, French J, Screaton NJ, Foweraker J, Condliffe A, Haworth CS, et al.** Nontuberculous mycobacteria in bronchiectasis: Prevalence and patient characteristics. Eur Respir J 2006; 28(6):1204-10.

25- **Palwatwichai A, Chaoprasong C, Vattanathum A, Wongsa A, Jatakanon A.** Clinical, laboratory findings and microbiologic characterization of bronchiectasis in Thai patients. Respirology 2002; 7(1):63-6.

26- **Pieters A, Bakker M, Hoek RAS, Altenburg J, van Westreenen M, Aerts JGJV, et al.** Predicting factors for chronic colonization of Pseudomonas aeruginosa in bronchiectasis. Eur J Clin Microbiol Infect Dis 2019; 38(12):2299-2304.

27- **Sharif N, Baig MS, Sharif S, Irfan M.** Etiology, Clinical, Radiological, and Microbiological Profile of Patients with Non-cystic Fibrosis Bronchiectasis at a Tertiary Care Hospital of Pakistan. Cureus 2020; 12(3):e7208.

28- **Dhar R, Singh S, Talwar D, Mohan M, Tripathi SK, Swarnakar R, et al.** Bronchiectasis in India: results from the

European multicentre bronchiectasis audit and research collaboration (EMBARC) and respiratory research network of India registry. Lancet Glob Health 2019; 7(9): e1269-e1279.

29- **Buscot M, Pottier H, Marquette CH, Leroy S.** Phenotyping Adults with Non-Cystic Fibrosis Bronchiectasis: A 10-Year Cohort Study in a French Regional University Hospital Center. Respiration 2016; 92(1):1-8.

30- **McDonnell MJ, Jary HR, Perry A, MacFarlane JG, Hester KL, Small T, et al.** Non cystic fibrosis bronchiectasis: A longitudinal retrospective observational cohort study of Pseudomonas persistence and resistance. Respir Med 2015; 109(6):716-26.

31- **Guan WJ, Gao YH, Xu G, Lin ZY, Tang Y, Li HM, et al.** Aetiology of bronchiectasis in Guangzhou, southern China. Respirology 2015; 20(5):739-48.

32- **King PT, Holdsworth SR, Freezer NJ, Villanueva E, Holmes PW.** Microbiologic follow-up study in adult bronchiectasis. Respir Med 2007; 101(8):1633-8.

33- **Dimakou K, Triantafillidou C, Toumbis M, Tsikritsaki K, Malagari K, Bakakos P.** Non CF-bronchiectasis: Aetiologic approach, clinical, radiological, microbiological and functional profile in 277 patients. Respir Med 2016; 116:1-7.

34- **Aksamit TR, O'Donnell AE, Barker A, Olivier KN, Winthrop KL, Daniels MLA, et al.** Bronchiectasis Research Registry Consortium. Adult Patients With Bronchiectasis: A First Look at the US Bronchiectasis Research Registry. Chest 2017; 151(5):982-992.

35- **Hsieh MH, Lin CY, Wang CY, Fang YF, Lo YL, Lin SM, et al**. Impact of concomitant nontuberculous mycobacteria and Pseudomonas aeruginosa isolates in non-cystic fibrosis bronchiectasis. Infect Drug Resist 2018; 11:1137-1143.

36- **Park J, Kim S, Lee YJ, Park JS, Cho YJ, Yoon HI, et al.** Factors associated with radiologic progression of non-cystic fibrosis bronchiectasis during long-term follow-up. Respirology 2016; 21(6):1049-54.

37- **Kadowaki T, Yano S, Wakabayashi K, Kobayashi K, Ishikawa S, Kimura M, et al.** An analysis of etiology, causal pathogens, imaging patterns, and treatment of Japanese patients with bronchiectasis. Respir Investig 2015; 53(1):37-44.

38- **Blackall SR, Hong JB, King P, Wong C, Einsiedel L, Rémond MGW, et al.** Bronchiectasis in indigenous and non-indigenous residents of Australia and New Zealand. Respirology 2018; 23(8):743-749.

39- **Faverio P, Stainer A, Bonaiti G, Zucchetti SC, Simonetta E, Lapadula G, et al.** Characterizing non-tuberculous mycobacteria infection in bronchiectasis. Int J Mol Sci 2016; 17(11): 1913.

40- **Zhou Y, Mu W, Zhang J, Wen SW, Pakhale S.** Global prevalence of non-tuberculous mycobacteria in adults with non-cystic fibrosis bronchiectasis 2006–2021: a systematic review and meta-analysis. BMJ open 2022; 12(8): e055672.

41- **Jamal F, Hammer MM.** Nontuberculous Mycobacterial Infections. Radiol Clin North Am 2022; 60(3):399-408.

42- **Chandrasekaran R, Mac Aogáin M, Chalmers JD, Elborn SJ, Chotirmall SH.** Geographic variation in the aetiology, epidemiology and microbiology of bronchiectasis. BMC Pulm Med 2018; 18(1): 83.

43- **Kotilainen H, Valtonen V, Tukiainen P, Poussa T, Eskola J, Järvinen A.** Prognostic

value of American Thoracic Society criteria for non-tuberculous mycobacterial disease: a retrospective analysis of 120 cases with four years of follow-up. Scand J Infect Dis 2013; 45(3):194-202.

44- **Zhu YN, Xie JQ, He XW, Peng B, Wang CC, Zhang GJ, et al.** Prevalence and clinical characteristics of nontuberculous mycobacteria in patients with bronchiectasis: a systematic review and meta-analysis. Respiration 2021; 100(12): 1218-1229.

45- **Stephenson D, Perry A, Appleby MR, Lee D, Davison J, Johnston A, et al**. An evaluation of methods for the isolation of nontuberculous mycobacteria from patients with cystic fibrosis, bronchiectasis and patients assessed for lung transplantation. BMC Pulm Med 2019; 19(1): 19.

46- **Kamada K, Yoshida A, Iguchi S, Arai Y, Uzawa Y, Konno S, et al.** Geographical distribution and regional differences in 532 clinical isolates of rapidly growing mycobacterial species in Japan. Sci Rep 2021; 11(1): 4960.

47- **Dadheech M, Malhotra AG, Patel S, Singh J, Khadanga S, Khurana A, et al.** Molecular Identification of Non-tuberculous Mycobacteria in Suspected Tuberculosis Cases in Central India. Cureus 2023; 15(6): e39992.

48- **Kwak N, Lee JH, Kim HJ, Kim SA, Yim JJ.** New-onset nontuberculous mycobacterial pulmonary disease in bronchiectasis: tracking the clinical and radiographic changes. BMC Pulm Med 2020; 20(1): 293.

49- **Chu H, Zhao L, Xiao H, Zhang Z, Zhang J, Gui T, et al.** Prevalence of nontuberculous mycobacteria in patients with bronchiectasis: a meta-analysis. Arch Med Sci 2014; 10(4): 661-668. doi: 10.5114/aoms.2014.44857.

50- **Wickremasinghe M, Ozerovitch LJ, Davies G, Wodehouse T, Chadwick MV, Abdallah S, et al.** Non-tuberculous mycobacteria in patients with bronchiectasis. Thorax 2005; 60(12): 1045-1051.

51- **Metersky M, Chalmers J.** Bronchiectasis insanity: Doing the same thing over and over again and expecting different results? F1000Res 2019; 8:F1000 Faculty Rev-293.

52- **Izumi K, Morimoto K, Hasegawa N, Uchimura K, Kawatsu L, Ato M, et al.** Epidemiology of Adults and Children Treated for Nontuberculous Mycobacterial Pulmonary Disease in Japan. Ann Am Thorac Soc 2019; 16(3):341-347.

53- **Mirsaeidi M, Hadid W, Ericsoussi B, Rodgers D, Sadikot RT.** Non-tuberculous mycobacterial disease is common in patients with non-cystic fibrosis bronchiectasis. Int J Infect Dis 2013; 17(11): e1000-e1004.

54- **López-Roa P, Aznar E, Cacho J, Cogollos-Agruña R, Domingo D, García-Arata MI, et al.** Epidemiology of Non-Tuberculous Mycobacteria isolated from clinical specimens in Madrid, Spain, from 2013 to 2017. Eur J Clin Microbiol Infect Dis 2020; 39(6):1089-1094.

55- **Han XY, Tarrand JJ, Infante R, Jacobson KL, Truong M.** Clinical significance and epidemiologic analyses of Mycobacterium avium and Mycobacterium intracellulare among patients without AIDS. J Clin Microbiol 2005; 43(9): 4407-4412.

56- **Modrá H, Ulmann V, Caha J, Hübelová D, Konečný O, Svobodová J, et al.** Socio-Economic and Environmental Factors Related to Spatial Differences in Human Non-Tuberculous Mycobacterial Diseases in the Czech Republic. Int J Environ Res Public Health 2019;16(20):3969.

57- **Chai J, Han X, Mei Q, Liu T, Walline JH, Xu J, et al.** Clinical Characteristics and Mortality of Non-tuberculous Mycobacterial Infection in Immunocompromised vs. Immunocompetent Hosts. Front Med (Lausanne) 2022; 9:884446.

Elmongi M, Elkenawy MF, Elhadidy TA, Elnagar RM. Nontuberculous mycobacterial infection in patients with bronchiectasis. A single-center study. Microbes Infect Dis 2024; 5(1): 155-167.