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Epidemiological and clinical characteristics of nontuberculous mycobacterial infections: A retrospective female cohort study in an Italian population

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ABSTRACT

Objectives: This study aims to assess the characteristics and treatment outcomes of patients diagnosed with nontuberculous mycobacteria (NTM) diseases at the Infectious Diseases Unit of ARNAS Garibaldi Hospital in Catania, Italy, focusing on demographics, clinical features, and treatment effectiveness.
Methods: We conducted a retrospective observational study of 10 patients diagnosed with NTM diseases between 2019 and 2021. Data was collected from electronic medical records, including demographic information, comorbidities, treatment modalities, and outcomes. The study utilized descriptive statistics to analyze continuous and categorical variables. Treatment regimens were based on individual patient needs, incorporating a combination of antibiotics.
Results: The median age of the patients was 55.44 years, all female, predominantly suffering from pulmonary NTM diseases. *Mycobacterium intracellulare* was the most common pathogen. Common comorbidities included COPD, bronchiectasis, GERD, and hypovitaminosis D. Patients showed symptoms like fever, cough, and asthenia. The treatment regimens were diverse, with macrolides, rifampicin, and ethambutol forming the core. Adverse effects were noted in 40 % of patients, including gastrointestinal and neurological disorders. All patients achieved microbiological cure, with 60 % showing clinical improvement and 36 % radiological improvement.
Conclusion: The study highlights the complexity of diagnosing and treating NTM diseases, emphasizing the need for personalized treatment plans and vigilant monitoring of adverse effects. Despite achieving microbiological cure, challenges remain in achieving complete clinical and radiological resolution. Further research is needed to enhance the understanding and management of NTM diseases, particularly in diverse populations.

Introduction

Nontuberculous mycobacteria (NTM) are a diverse group of opportunistic pathogens that are ubiquitous in the environment, including in soil, dust, and water sources. These organisms are not classified as part of the *Mycobacterium tuberculosis* complex or *M. leprae*, the bacteria responsible for tuberculosis and leprosy, respectively [1]. While NTM diseases have been recognized since the late 19th century, they were not widely acknowledged as significant causes of human disease until the second half of the 20th century [2].

The epidemiology of NTM is complex and varies regionally, but the incidence of NTM pulmonary disease (NTM-PD) has been observed to be increasing globally. This trend is particularly evident in North America and parts of the world where the rates of tuberculosis have been decreasing [3,4]. Among the NTM species, the *Mycobacterium avium* complex (MAC) is the most common cause of NTM-PD, found in up to 72

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% of patients with NTM-positive sputum cultures. However, the incidence of *Mycobacterium abscessus*, a particularly antibiotic-resistant NTM species, has also been on the rise, posing major clinical and therapeutic issues [5].

NTM infections can manifest in a variety of clinical presentations, affecting both immunocompetent and immunocompromised individuals [6,7]. While the diseases caused by NTM are generally considered less virulent than those caused by *M. tuberculosis*, they can nonetheless result in significant morbidity and are a persistent burden that remains undiagnosed in many suspected cases. The increasing attention towards NTM infections in recent years has been due to their clinical diversity, ranging from pulmonary to extrapulmonary infections, the latter including skin, soft tissue, and sterile site infections [8].

Given the complexity and the rising trend of NTM diseases, understanding their epidemiology and impact on human health is essential. This includes recognizing the shifting patterns of species prevalence, the challenges in diagnosing and treating these infections, and the implications for public health strategies [9–11].

Materials and methods

We conducted a retrospective observational study of patients diagnosed with NTM diseases at the Infectious Diseases Unit of ARNAS Garibaldi Hospital in Catania, Italy. We included patients diagnosed with NTM diseases between 2019 and 2021, whose treatment was completed prior to the initiation of this study. The data was collected from electronic medical records from a single healthcare center. The demographic and clinical data of patients were retrieved from the healthcare center's database. Variables collected included age, gender, comorbidity conditions, treatment modalities, and outcomes. Additionally, we documented radiological patterns, symptoms at admission, the presence of previous pulmonary comorbidities, and any reported adverse effects during treatment. For the classification of NTM species, we referred to laboratory results of culture and microbial analysis. The localization of the NTM diseases was categorized as lung or other than lung involvement. The antibiotic treatment regimens were extracted from the patients' medical records. These included combinations of macrolides, rifampicin, ethambutol, aminoglycosides, tetracyclines, linezolid, clofazimine, and moxifloxacin, tailored according to individual cases. The study was approved by the Institutional Review Board (IRB) of the healthcare center from which the data was sourced. Patient confidentiality was maintained by de-identifying personal information in accordance with the Health Insurance Portability and Accountability Act (HIPAA) regulations. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request, subject to compliance with ethical regulations and privacy laws.

Statistical analysis

Statistical descriptive analyses were performed using the Statistical Package for Social Sciences (version 29, SPSS Inc., Chicago, Illinois). Continuous variables were expressed as mean ± standard deviation (SD) if normally distributed, or as median with interquartile range (IQR) for non-normally distributed data, as assessed by the Shapiro-Wilk test. Categorical variables were summarized as frequencies and percentages. The demographic and clinical characteristics of the participants, including age, gender distribution, comorbid conditions, treatment modalities, and outcomes, were tabulated and examined. We generated frequency distributions for categorical data to understand the prevalence of various attributes within the sample. Cross-tabulations were utilized to explore potential associations between categorical variables. For continuous variables, we provided measures of central tendency and dispersion, as well as confidence intervals where appropriate. Histograms and boxplots were employed to visually assess the distribution of quantitative data and identify potential outliers.

Results

We retrospectively analyzed data from 10 patients diagnosed with NTM diseases between 2019 and 2021, whose treatment concluded prior to data collection. The median age was 55.44 ± 12.74 years, ranging from 35 to 79, all of whom were female and Italian.

Among the patients, 9 had pulmonary NTM diseases, and 1 had lymphnode involvement. Radiologically, of the lung isolates, 5 (55.6 %) patients exhibited a fibro-cavitary pattern, and 4 (44.4 %) had a nodular pattern. The study included 6 patients with *M. intracellulare* infections, and the other mycobacteria remained consistent with 2 cases of *M. chelonae* and 1 case each of *M. scrofulaceum*, and *M. avium*, as detailed in Table 1.

All patients were diagnosed with two positive culture of sputum specimens or one positive culture of broncho alveolar lavage fluid (BAL) / washings; as regards the patient with lymphnode involvement, the diagnosis was made by histopathological and microbiological (culture on tissue biopsy) findings.

Pulmonary comorbidities were present in 70 % (7) of the patients, and COPD was diagnosed in 30 % (3). Five (50 %) patients had bronchiectasis, and 5 patients suffered from gastroesophageal reflux disease (GERD). Two patients had osteoporosis, whereas 2 patients suffered from hypovitaminosis D.

Fever was present in 80 % (8) of patients, cough in 100 % (10), and asthenia in 50 % (5). Weight loss was reported in 20 % (2) of patients. None of our patients suffered by tuberculosis in the past and none of them was HIV positive. Only one patient had HBeAg negative chronic HBV infection.

All patients received a combination of antibiotics, with macrolides, rifampicin, and ethambutol as core treatments. Additional drugs used in treatment, such as aminoglycosides, tetracyclines, linezolid, clofazimine, and moxifloxacin, were tailored to individual cases, as summarized in Table 2. One patient received 5 months of liposomal amikacin as rescue therapy for previous treatment failure.

Adverse effects during treatment were noted in 40 % (4) of the patients. These included hypertransaminasemia and peripheral neurological disorders in one patient each, nausea in 20 % (2), and vomiting in 10 % (1). One patient developed anxious disorder, which was attributed to moxifloxacin.

The mean total length of treatment (TLOT) is approximately 22.9 months, with a standard deviation of about 7.75 months.

As regards patients 3 and 8, affected by *M. chelonae* infections, since their treatment was challenging and had been changed several times due to intolerance and adverse events, their regimens are graphically summarized in Fig. 1 and Fig. 2.

All patients with pulmonary NTM achieved microbiological cure with negative culture performed during treatment administration; 6 patients achieved clinical cure with symptoms amelioration, whereas persistent cough remained in 4 patients. 2 patients underwent lobectomy to improve clinical outcomes, 1 patient started respiratory physiotherapy. Only 4 (36 %) out of 9 patients with pulmonary NTM achieved radiological improvement. One patient showed *P. aeruginosa* lung colonization. The patient with lymphnodes localization resolved his symptoms with surgical intervention.

Discussion

This retrospective observational study aimed to elucidate the characteristics and treatment outcomes of a group of patients diagnosed with and treated for NTM diseases at our hospital.

Our cohort included 10 Italian females with a median age of 55.44 years, in line with a potential gender and age-related predisposition in NTM disease occurrence. Recent studies suggest a gender-based predisposition in the incidence of NTM diseases, particularly in patients with non-cystic fibrosis bronchiectasis [12]. These studies have found a higher prevalence of NTM in elderly female non-smoking patients with

Table 1
Demographic and clinical characteristics of the study population,.

Patient	Species	Site	Radiological pattern	Age	Sex	Smoke	Symptoms (admission)	Comorbidities or Predisposing factors
1	*M. intracellulare*	L	FC	60	F	Yes	Fever, Cough,	Pnx, Bronchiectasic
2	*M. scrofulaceum*	L	N	58	F	Yes	Asthenia, Cough, Fever	Bronchiectasis, Thrombophilia congenital
3	*M. chelonae*	L	N	51	F	No	Cough, Asthenia	Recurrent bronchitis, GERD
4	*M. intracellulare*	LY	/	41	F	Unknown	Non-tender oval swelling in axillary right region	None
5	*M. intracellulare*	L	FC	60	F	Ex-smoker	Cough, Fever, Asthenia	CD, GERD, HBV infection, osteoporosis,
6	*M. intracellulare*	L	FC	35	F	No	Cough	GERD, ipovitamonosis D, thyroiditis, gastric adenomas
7	*M. intracellulare*	L	N	62	F	No	Cough, Fever	COPD, bronchiectasis, Aspergillosis
8	*M. chelonae*	L	N	53	F	Unknown	Weight Loss, Cough, Fever, Asthenia	COPD, bronchiectasis, Neoplasm (mammalian), GERD
9	*M. avium*	L	FC	79	F	Yes	Weight Loss, Cough, Fever, Asthenia	COPD, pleuritis, CD, osteoporosis, bronchiectasis
10	*M. intracellulare*	L	FC	48	F	Yes	Cough, Fever, Asthenia	GERD, Hypovitaminosis D, thyroiditis

PNX: pneumothorax; COPD: Chronic obstructive pulmonary disease; CD: cardiac disease; L: lungs; LY: lymphnode; N: nodular; FC: fibro-cavitary; GERD: gastro-esophageal reflux disease

Table 2
Performed nontuberculous mycobacteria treatment regimens.

Patient	Species	Combination	Dose (mg)	Frequency	TLOT (months)	Outcome	ADR
1	*M. intracellulare*	clarithromycin	500	Bid	24	M, C	None
		ethambutol	800	Qd			
		rifampicin	450	Qd			
2	*M. scrofulaceum*	clarithromycin	500	Bid	15	M (persistent cough)	None
		rifampicin	600	Qd			
		ethambutol	800	Qd			
3	*M. chelonae*	clarithromycin/	500	Bid	33	M (persistent cough)	Peripheral neurological disorders, anxious syndrome
		azithromycin	500	3/w			
		rifampicin	600	Qd			
		ethambutol	800	Qd			
		amikacin	750	3/w			
		doxycycline	100	Bid			
		linezolid	300	Qd			
		clofazimine	100	Qd			
		moxifloxacin	400	Qd			
4	*M. intracellulare*	azithromycin	500	3/w	12	C (surgical removal of lymphnode)	None
		rifampicin	600	3/w			
		ethambutol	1400	3/w			
5	*M. intracellulare*	clarithromycin	500	Bid	19	M, C, R	Hypertransaminasemia,nausea
		rifampicin	600	Qd			
		clofazimine (1 month)	200	Qd			
		moxifloxacin	400	Qd			
6	*M. intracellulare*	clarithromycin	500	Bid	25	M, C, R (VATS lobectomy)	None
		ethambutol	800	Qd			
		rifampicin	600	Qd			
7	*M. intracellulare*	clarithromycin	500	Bid-3/w	19	M(persistent cough)	None
		ethambutol	800	3/w			
		rifampicin	600	3/w			
8	*M. chelonae*	azithromycin	500	Qd	28	M (persistent cough and *Pseudomonas aeruginosa* colonization)	Vomit, nausea
		/clarithromycin	500	Bid			
		amikacin/	1000	3/w			
		tobramycin	200	Qd			
		doxycycline	200	Qd			
		linezolid	300	Qd			
		clofazimine	100	Qd			
		moxifloxacin	400	Qd			
9	*M. avium*	azithromycin	250	Qd	18	M, C, R(respiratory physiotherapy)	None
		rifampicin	450	Qd			
		ethambutol	600	Qd			
		amikacin	500	3/w			
10	*M. intracellulare*	rifampicin	600	Qd	36	M, C, R(VATS lobectomy)	None
		ethambutol	1000	Qd			
		clarithromycin	500	Bid-3/w			
		clofazimine	100	Qd			
		ALIS	590	Qd			

TLOT: Total length of treatment; ADR: adverse drug reactions; Qd: quaque die (every day); Bid: Bis in die; 3/w: three times a week; M: microbiological cure; R: radiological improvement; C: clinical cure; F: treatment failure; ALIS: Amikacin liposome inhalation suspension; VATS: Video-assisted thoracoscopic surgery

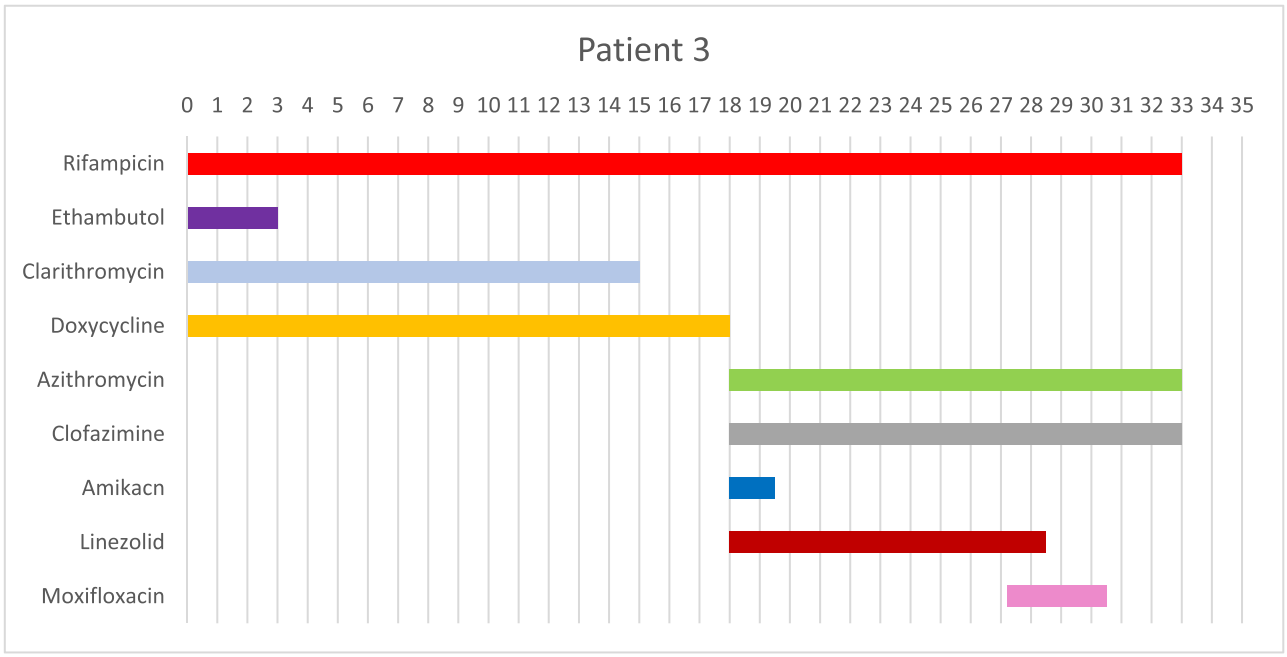

Fig. 1. Antibiotic treatment regimen of patient 3 with *M. chelonae* infection; x-axis reports duration in months.

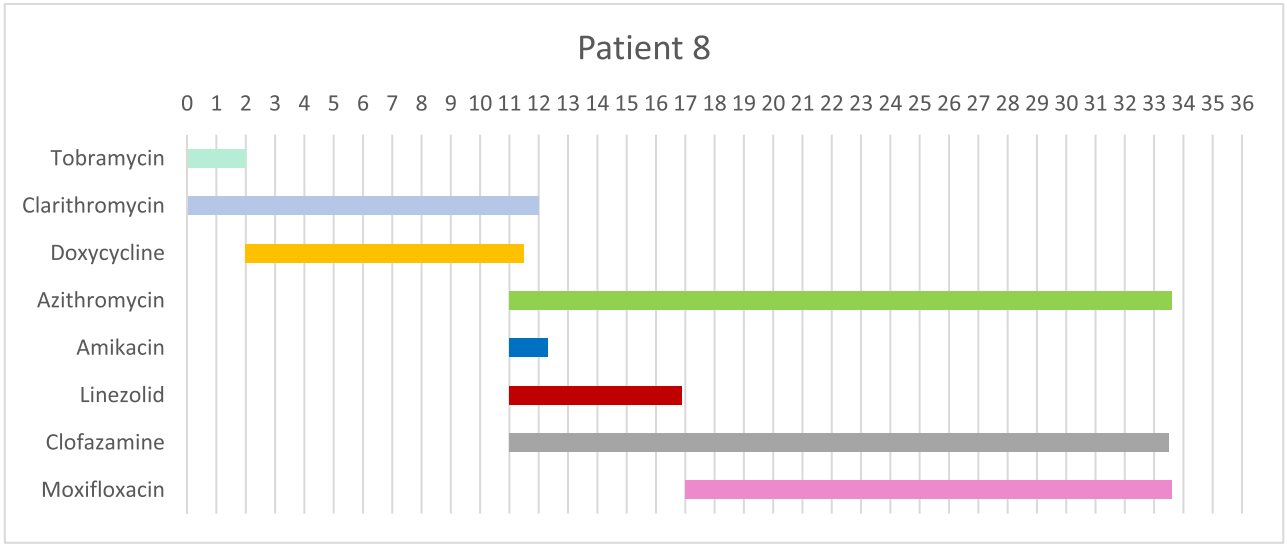

Fig. 2. Antibiotic treatment regimen of patient 8 with *M. chelonae* infection; x-axis reports duration in months.

slender body habitus [13]. However, the mechanisms of causality of these gender differences and morpho-phenotypes remain to be understood.

The predominance of lung involvement in our cohort (9 out of 10 cases) is consistent with existing literature suggesting the lungs as the primary site for NTM infections [14]. The distribution of radiological patterns, with equal prevalence of nodular and fibro-cavitary patterns, is not common and maybe this is due to the small sample size since the nodular bronchiectasic pattern is generally more represented, especially in female patients with newly diagnosed MAC infections [15,16].

In our study, low vitamin D levels and osteoporosis were observed in four patients. This finding aligns with the research conducted by Jeon and colleagues, which demonstrated a higher prevalence of severe vitamin D deficiency in patients with NTM lung disease than in controls. Their study further identified severe vitamin D deficiency as an independent factor associated with NTM lung disease [17].

Half of patients in our cohort suffered from GERD, in line with the results of Kim et al. [18] who highlighted that patients with GERD had a significantly higher incidence of NTM PD compared to matched patients without GERD. The study also identified older age and bronchiectasis as risk factors for NTM PD among patients with GERD. Similarly, Koh and colleagues [19] explored the prevalence of GERD in patients with nodular bronchiectasic form of NTM lung disease, finding that 26 % of these patients had GERD. Interestingly, a significant portion of these patients did not exhibit typical GERD symptoms. The study further noted that patients with GERD were more likely to have positive sputum smears for acid-fast bacilli, suggesting a possible link between GERD and an increased mycobacterial burden.

As reported by Italian IRENE registry [20], the species distribution in our cohort, led by *M. intracellulare*, reflects global and Italian trends but also underlines the necessity for regional surveillance to monitor species-specific prevalence and resistance patterns [3].

Major risk factors for NTM pulmonary diseases include the presence of structural lung diseases (COPD, bronchiectasis, cystic fibrosis, history of previous tuberculosis, etc.) and congenital or acquired immunosuppression (primary immunodeficiency, HIV infection, transplantation, etc.). All our patients were immunocompetent, none of them had HIV infections, none of them had previous pulmonary tuberculosis. Furthermore, all of them were screened for viral hepatitis and sexually transmitted diseases. Notably, the high proportion of patients with pre-existing pulmonary conditions, particularly COPD and bronchiectasis, underscores the need for heightened clinical vigilance in such populations [21]. Concerning clinical presentation, as reported in other studies, fever, persistent/chronic cough and asthenia are common manifestation of NTM-PD, as well as haemoptysis, shortness of breath, and chest pain. These aspecific symptoms along with the presence of other conditions that could explain account for the cumbersome and often delayed diagnosis.

Regarding the diagnostic criteria, all our patients with pulmonary diseases underwent respiratory secretion culture. NAAT was performed on positive cultures to rapidly identify NTM species. Our lab does not perform NTM antibiotic susceptibility test. The patient with lymph nodes involvement was diagnosed by histopathological criteria and positive culture on tissue biopsy. It is worth to precise that for diagnosing NTM-PD, the microbiological criteria include either two positive sputum culture results or one positive bronchoalveolar lavage fluid culture, as stated by international guidelines [22].

For the treatment of pulmonary NTM disease due to MAC or *M. scrofulaceum*, the ATS/ERS/ESCMID/IDSA clinical practice guideline [22] recommends a three-drug regimen that includes a macrolide, azithromycin or clarithromycin, along with ethambutol and rifampicin. For severe cases, such as cavitary disease or extensive bronchiectasis, adding parenteral amikacin may be beneficial. The use of clofazimine, although with discordant literature data, is justified by its antimycobacterial effect together with its immunomodulating action [23]. One of our patients had to stop clofazimine after one month due to nausea.

In our study, 5 out of 8 patients with MAC or *M. scrofulaceum* infections were treated with three-drug regimen (rifampin, macrolide, and ethambutol); the remaining 3 patients received the same regimen with the addition of clofazimine and amikacin, for two and for one of them, respectively.

One patient received amikacin liposome inhalation suspension (ALIS) 590 mg/8.4 mL/die for 5 months, since she met inclusion criteria to receive ALIS as compassionate use.

The management of *M. chelonae* infections presents unique challenges that are multifaceted. Firstly, *M. chelonae* is known for its robust resistance to conventional antibiotics, making the selection of an effective treatment regimen difficult. The study reflects this complexity, as patients required an array of antibiotics that were frequently modified due to intolerance and adverse reactions. Fig. 1 and Fig. 2 showed the complexity of antibiotics which were changed mainly due to adverse reactions.

For serious cases of *M. chelonae* pulmonary diseases a three-drug regimen is recommended [24,25]. The therapeutic approach usually includes one or two intravenous medications, often with tobramycin as the aminoglycoside of choice, during the initial phase, which can last from 4 to 16 weeks. Oral medications are also prescribed, including a macrolide that has shown in-vitro efficacy against the organism, and treatment is sustained for at least one year following the achievement of a culture-negative status to ensure comprehensive management of the condition [26,27]. However, the current body of literature does not provide sufficient evidence to establish a link between the specific drug treatment regimen for *M. chelonae* pulmonary disease and the likelihood of a successful treatment outcome.

These challenges are compounded by *M. chelonae* ability to form biofilms, which further reduces the efficacy of antimicrobial agents. Moreover, the variability in antibiotic penetration in lung tissue can affect the outcomes of treatment for pulmonary diseases. The extended duration of therapy, often necessary for managing *M. chelonae*, increases the risk of drug toxicity and patient non-compliance, which are significant barriers to successful treatment. This aspect is especially pertinent considering the adverse reactions reported in our study, emphasizing the need for close monitoring and potential strategies to mitigate these effects, such as drug desensitization protocols or the use of alternative antibiotics with lower toxicity profiles. The two patients we reported with *M. chelonae* infection did not show any improvement in their clinical symptoms or in radiological assessments, although they did achieve a microbiological cure.

The adverse effects reported, including hypertransaminasemia, peripheral neurological disorders, nausea, and vomiting, are known complications of the antibiotics used and highlight the challenges in managing the tolerability of NTM treatment options [28]. Hepatic toxicities are the primary concern, as additional medications may lead to hepatitis (e.g., rifampin, ethambutol), along with gastrointestinal adverse events. For patients receiving macrolides, cardiac function should be carefully evaluated using electrocardiograms, particularly for QT prolongation. Furthermore, renal function should be frequently assessed in those receiving parenteral aminoglycosides.

Antibiotic treatment duration was extended up to 36 months and it was higher for patients with *M. chelonae* infections, to prove the difficulty in treating this type of infection. As suggested by guidelines, NTM treatment for pulmonary localizations should be protracted for at least 12 months after culture conversion. The management of NTM lymphadenitis typically involves the surgical removal of the affected lymph nodes. Studies have shown that this method is more effective in achieving higher cure rates compared to pharmacological treatment. For cases where the surgical removal is not comprehensive or when lymphadenitis reappears post-surgery, antibiotics or a watchful waiting strategy might be considered. Although the ideal treatment approach for MAC lymphadenitis has not been conclusively established, a combined drug regimen that typically includes clarithromycin, rifampin, and possibly ethambutol, could prove advantageous [29].

All patients achieved microbiological cure with negative cultures. Unfortunately, only six patients experienced clinical cure with resolution of symptoms. The remaining four patients continued to have a persistent cough despite the respiratory culture conversion. Significantly, two out of five patients with a fibrocavitary pattern underwent lobectomy to enhance their clinical status, in accordance with guidelines.

From a radiological perspective, we cannot declare a cure as the pulmonary lesions and the clinical presentations resulting from the infection, or those that the infection complicates, are not capable of regression but can only exhibit moderate improvement. Determining the precise impact of the infection on pulmonary damage, and conversely, the extent to which pre-existing lung damage has affected the infection, is often challenging. Nevertheless, 4 patients achieved radiological amelioration.

The limitations of our study include its retrospective nature, the small sample size, and single-center design, which may affect the generalizability of the findings. Additionally, the absence of HIV-infected individuals may not accurately represent the full spectrum of NTM disease severity seen in immunocompromised populations [30].

Results by two bigger Italian studies have already been published, Laura Rindi and Carlo Garzelli [31], analyzed the epidemiology of NTM infections in Tuscany, Italy, over an 11-year period. The researchers collected data from 42,055 clinical specimens finding a significant increase in NTM isolates from 2004 to 2014, with a total of 147 patients testing positive for NTM. The most common NTM species were *M. avium* (41.5 %), *M. intracellulare* (14.3 %), and *M. gordonae* (11.6 %). The majority of isolates (76.2 %) were from respiratory specimens, primarily affecting older adults over 60 years. Aliberti et al. [32], retrospectively evaluated clinical outcomes in patients treated for NTM-PD over ten years in Milan, Italy. The study included 170 patients, with NTM-PD primarily caused by MAC (71.2 %), *M. kansasii* (9.4 %), and *M. xenopi*

(7.1 %). The median follow-up was 31 months. Adverse events occurred in 37.6 % of patients, leading to treatment discontinuation in 13.5 %. Overall, 35.3 % of patients experienced unsuccessful treatment outcomes, including recurrence, re-infection, and treatment failure. Although smaller and under-powered, the study we reported aligns with these previous larger studies in terms of microbiological findings, treatment success rate, and adverse events, highlights the ongoing clinical and radiological challenges, mirroring the broader issues identified in the larger studies.

Furthermore, our lab did not perform antibiotic susceptibility testing for NTM which could be helpful to treat challenging infections, such as those by *M. chelonae*.

In conclusion, our findings contribute to the growing body of evidence on the epidemiology and treatment challenges of NTM diseases. They underscore the importance of individualized treatment regimens and the need for ongoing monitoring of adverse effects. Further research with larger, multicenter cohorts is necessary to validate these findings and to explore the impact of NTM diseases on diverse populations. Future studies should also aim to establish the genetic, environmental, and clinical factors that contribute to the susceptibility and outcomes of NTM infections, thereby informing public health strategies and clinical guidelines.

Additionally, it is of fundamental importance to exclude causes of immunodeficiency, whether congenital or acquired, in patients affected by NTM; carefully assess the risk/benefit ratio of the treatment, considering the side effects of the drugs, interactions with other medications, the duration of therapy, and patient compliance; and consider any concurrent or adjuvant treatments such as surgery or respiratory physiotherapy.

Lastly, antibiotic susceptibility testing is crucial for guiding effective treatment strategies and can significantly impact the management of those infections caused by inherently resistant organisms like *M. chelonae*. The establishment of standardized, reliable, and accessible susceptibility testing methods for NTM would provide clinicians with the necessary information to choose the most appropriate antibiotics, potentially improving patient outcomes. Additionally, regular susceptibility testing could help in the surveillance of resistance trends, which is essential for updating treatment guidelines and informing public health interventions. The development of rapid susceptibility testing methods would be especially beneficial in reducing the time to effective treatment, which is often prolonged in NTM infections due to the slow-growing nature of these bacteria.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Comitato Etico Catania 2 (protocol code 101/CECT2 – approved on 18th April 2023).

Ethical Approval statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Comitato Etico Catania 2 (protocol code 101/CECT2 – approved on 18th April 2023). Written informed consent was obtained from all the patients involved in the study.

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CRediT authorship contribution statement

Conceptualization: AM and MPR; Data curation: MG; Investigation: GS, VM and AL; Methodology: SS; Roles/Writing - original draft: AM and MPR; Writing - review & editing: DB, BC and GN.

CRediT authorship contribution statement

Alessandro Libra: Writing – review & editing, Investigation, Data curation. **Serena Spampinato:** Investigation. **Dafne Bongiorno:** Supervision. **Bruno Cacopardo:** Writing – original draft, Supervision. **Giuseppe Nunnari:** Writing – review & editing, Supervision. **Andrea Marino:** Investigation, Conceptualization. **Michele Salvatore Paternò Raddusa:** Investigation, Conceptualization. **Maria Gussio:** Writing – original draft, Methodology, Investigation, Conceptualization. **Giuseppe Sangiorgio:** Supervision, Data curation. **Vittoria Moscatt:** Supervision, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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