Relationship between age, sex, geography and incidence of nontuberculous mycobacteria in Denmark from 1991 to 2022

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Shareable abstract (@ERSpublications)
Pulmonary NTM incidence rates have been increasing in Denmark from 1991 to 2022, particularly in older females. There are considerable geographical differences, with up to 40% higher rates in less-populated municipalities. https://bit.ly/4gNwfhk

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Abstract

Objectives We investigated age, sex and geographical differences in nontuberculous mycobacteria (NTM) incidence in Denmark.

Methods A nationwide register-based study of all patients with NTM isolates in Denmark from 1991 to 2022 based on centralised microbiological data from the International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark. A Poisson regression model was used to calculate incidence rates (IRs) and rate ratios (IRRs).

Results 4123 patients had NTM isolated for the first time. Their median age was 59 years (interquartile range 33–72), which increased over time. Males were younger than females. The proportion of females increased significantly over time. The type of NTM and patient age were closely associated. Pulmonary NTM isolation was increasingly common with higher age, while extrapulmonary NTM isolation was mainly seen in small children. Pulmonary NTM IRs were almost twice as high for females in 2008–2022 compared to 1991–2007 (IRR 1.9, 95% CI 1.7–2.1, p<0.001), with increases mainly seen in older age groups. The increase was less pronounced for males (IRR 1.3, 95% CI 1.1–1.4, p<0.001). There were considerable geographical differences, with age- and sex-adjusted NTM IRs being 10–40% higher in countryside, provincial and catchment municipalities than in the capital.

Conclusion Age, sex and geography are essential determinants in NTM epidemiology. We found that rates of pulmonary NTM have been increasing, particularly in older females, while changes for males were less pronounced. Finally, we observed considerable geographical differences in NTM IRs in Denmark, with higher rates in less populated municipalities.

Introduction

The epidemiology of nontuberculous mycobacteria (NTM) infections is influenced by patient age, sex, comorbidities and geographical location, among other factors [1–4]. Typically, infections are seen as either cervical lymph node infections in small children or as pulmonary infections in middle-aged to older individuals with chronic lung disease [5]. In some countries, higher rates of NTM infection have been reported in females [1, 2]. Prevalence, incidence and species distribution of NTM infections vary considerably between different regions of the world [3]. In most regions, prevalence and incidence rates of pulmonary NTM have been observed to increase [4]. The highest rates of NTM have been reported from East Asia, North America and Australia compared to Europe [3]. Within continents and countries,

differences have also been observed [5–9]. Overall, the epidemiology of NTM infections is complex and multifactorial, and age, sex and geography are essential determinants of the risk for NTM infection and disease. However, the understanding of these determinants as risk factors for NTM is still incomplete [10].

In this nationwide study including all patients with NTM isolates in Denmark over 32 years, we investigated age, sex and geographical differences in NTM incidence.

Methods

Study design

This is a nationwide register-based study of all patients with NTM isolates in Denmark from 1991 to 2022 based on centralised microbiological data from the International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark. In addition, publicly available population data from Statistics Denmark, including municipality data, were used [11].

Study setting

Denmark, a resource-rich industrialised Scandinavian country, has a population of approximately 5.9 million with a median age of 42 years and an equal distribution of males and females [11]. The Danish healthcare system is universal and taxpaid and based on the principles of free and equal access to healthcare for all citizens. The area of Denmark is 42 952 km^2, with 7300 km of bordering coastline and 1419 islands, of which only a small proportion is inhabited [11].

NTM data

Patients were included only on first occasion of NTM isolation. NTM were categorised by species or group as described by Tortoli *et al.* [12]. NTM species identification was performed as recently described [13]. Patients with multiple species isolated concurrently were excluded as well as patients with *Mycobacterium gordonae* isolates only, considering this pathogen an extremely rare cause of disease. Disease localisation was categorised as pulmonary, extrapulmonary or disseminated (if positive samples from both pulmonary and extrapulmonary localisations).

Statistical analyses

Characteristics of NTM isolation were presented by age, sex and type of municipality of residence. Categorical data were presented as numbers and proportions, and continuous data as medians with interquartile ranges (IQRs). A Poisson regression model was used to calculate incidence rates (IRs) of NTM isolation and incidence rate ratios (IRRs) with corresponding 95% confidence intervals to compare IRs between age groups, sexes, municipalities of residence and types of municipalities of residence, and different time periods. This model was also used to adjust IRRs for age groups and sex. Unadjusted IRs for each municipality in Denmark from 2008–2022 were depicted using the R package map*DK* (v. 0.3.0). For evaluations of geographical differences, only NTM and population data from 2008 to 2022 were used due to the availability of information on the area of patient residence and age and sex distributions in the 98 municipalities in Denmark, allowing for age and sex adjustment of estimates. The type of municipality of residence at isolation of NTM was categorised as either 1) capital, 2) metropolitan, 3) provincial, 4) catchment or 5) countryside with a decreasing number of places of employment and inhabitants in each municipality as defined by Statistics Denmark [14].

Ethical considerations

The Danish Data Protection Agency approved the study through the Department of Compliance, Statens Serum Institut, Copenhagen (22/00845). All patient data are presented as aggregates and anonymised.

Results

During the 32-year study period, 4123 patients had NTM isolated after exclusion of 896 patients with *M. gordonae* and 34 patients with multiple species.

Age

The median patient age was 59 years (IQR 33–72). The median age increased from 64 years (IQR 50–73) in 1991–2007 to 68 years (IQR 57–76) in 2008–2022 (Wilcoxon rank sum test, p<0.001). Males were younger than females (57 years, IQR 34–71 *versus* 61 years, IQR 30–74, p=0.002). The type of NTM and patient age were closely associated (figure 1). For instance, *M. avium* complex and *M. malmoense* had distinct bimodal age distributions. *M. avium* complex was dominant in all age groups (supplement 1). Pulmonary NTM isolation was more frequent in higher age groups, with an IR of 5.9 (95% CI 5.5–6.3) per 100 000 individuals for those older than 74 years (figure 2). In contrast, extrapulmonary NTM was more common among 0–14-year-olds (IR 2.0, 95% CI 1.9–2.2), particularly pronounced in 1- and

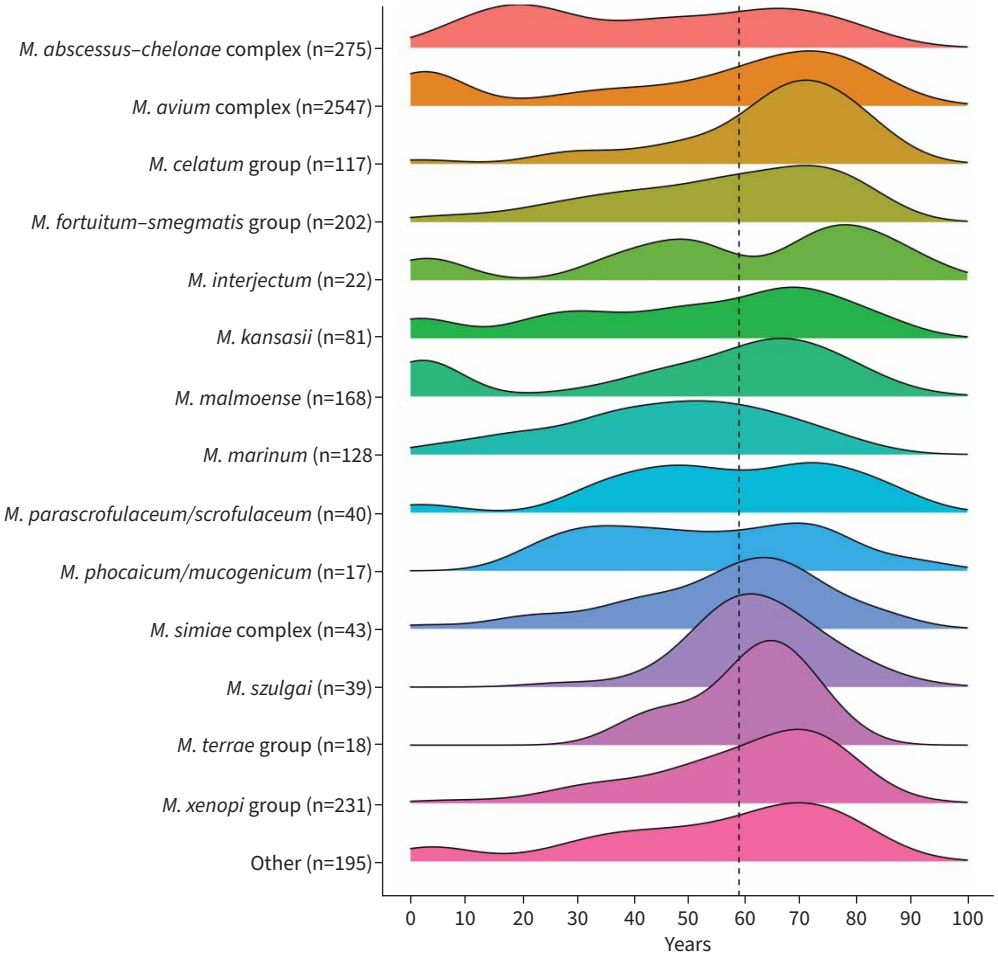

FIGURE 1 Ridge (density) plot of age distributions by most common nontuberculous mycobacteria in Demark from 1991 to 2022. The vertical black line (dashed) indicates the median age of all patients. "Other" was defined as *Mycobacterium* species and species with n<15 throughout the study period.

2-year-olds (supplement 2). For children aged 0–5 years, NTM IRs were higher during the winter (IRR 1.8, 95% CI 1.3–2.4, p<0.001) and spring months (IRR 1.5, 95% CI 1.1–2.1, p=0.007) compared to summer (2004–2022 due to availability of exact sample receival date).

Sex

A little less than half of the patients were females (n=1 997, 48%), but this proportion increased from 43% in 1991–2007 to 52% in 2008–2022 (Pearson's Chi-squared test, p<0.001). Males had a higher rates of disseminated and extrapulmonary NTM compared to females (table 1). *M. marinum*, *M. parascrofulaceum/scrofulaceum* and *M. szulgai* were more common among males. Males had higher NTM IRs than females in all age groups except for 0–14-year-olds (IRR 0.7, 95% CI 0.6–0.8, p<0.001) (table 1 and supplement 3). Unadjusted NTM IRs for females of all ages increased considerably over time (from 1.4 per 100 000 in 1991 to 2.9 in 2022) (figure 3). For sexes combined, age- and sex-adjusted NTM IRs were higher from 2008 to 2022 compared with from 1991 to 2007 (IRR 1.2, 95% CI 1.1–1.3, p<0.001). However, for males, age-adjusted overall NTM IRs in 2008–2022 did not increase compared to the earlier time period (IRR 1.0, 95% CI 0.9–1.1, p=0.877), whereas the rates for females increased considerably in comparison (IRR 1.5, 95% CI 1.4–1.7, p<0.001). NTM IRs of pulmonary isolation increased over time for both males (IRR 1.3, 95% CI 1.1–1.4) and females (IRR 1.9, 95% CI 1.7–2.1), while NTM IRs of extrapulmonary and disseminated isolation did not.

Geography

NTM IRs by age group, sex, disease localisation and species distribution of most common species, divided by type of municipality of residence, are presented in table 2. In less densely populated municipalities,

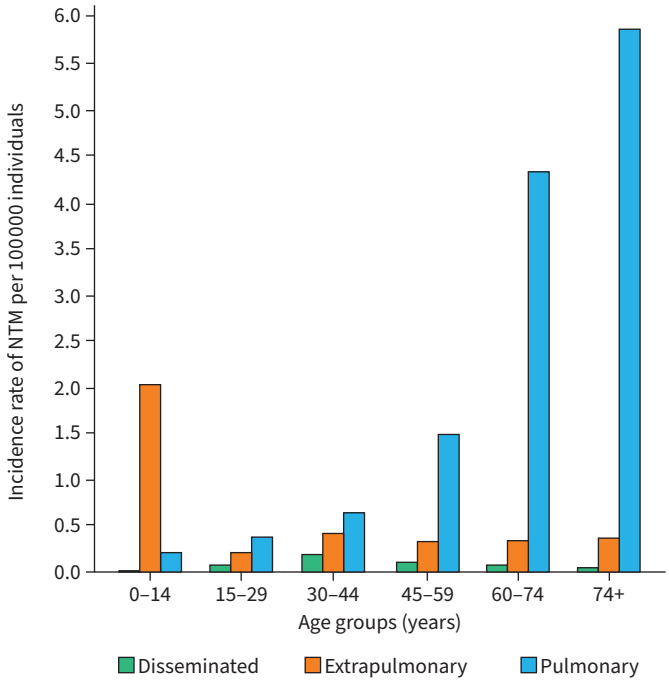

FIGURE 2 Incidence rates of nontuberculous mycobacteria (NTM) isolation per 100 000 individuals in Denmark from 1991 through 2022 by age group and disease localisation.

NTM IRs were generally higher among older patients and patients with pulmonary NTM compared to the capital. *M. avium* complex was also increasingly incident in more rural municipalities. NTM IRs varied between geographical areas by age and sex (supplement 4). Unadjusted IRs also varied considerably between different municipalities in Denmark (figure 4 and supplement 5). Age- and sex-adjusted NTM IRs were higher in the countryside (IRR 1.3, 95% CI 1.1–1.4, p<0.001), provincial (IRR 1.2, 95% CI 1.1–1.4, p<0.001) and catchment (IRR 1.2, 95% CI 1.1–1.4, p=0.005) municipalities compared to the capital municipality (figure 5). Unadjusted IRs of NTM for all types of municipalities increased gradually over time (supplement 6), but only IRs in the capital were higher in 2016–2022 compared with 2008–2015 (IRR 1.2, 95% CI 1.0–1.4, p=0.046) when adjusting for age and sex differences. This was seen only for females (IRR 1.4, 95% CI 1.1–1.8, p<0.001).

Discussion

In this nationwide study including all patients with NTM isolates in Denmark over 32 years, we found clear differences in NTM isolation by age, sex and geography. The type of NTM species and age were closely associated. Pulmonary NTM isolation rates increased substantially with higher age, while extrapulmonary NTM isolation was seen mainly in small children. NTM was more common in males than in females. Nevertheless, pulmonary NTM IRs almost doubled for females from 1991–2007 to 2008–2022, with increases mainly seen in older age groups, whereas rates among males only increased slightly. Finally, we found considerable geographical differences in NTM IRs, with IRs being 10–40% higher in countryside, provincial and catchment municipalities than in the capital.

Increasing NTM pulmonary disease rates with higher age is well-described [1, 5, 15–17]. We found some NTM had distinct bimodal age distributions (*M. avium* complex and *M. malmoense*), most likely representing cervical lymphadenitis in children and pulmonary infection in middle-aged and older patients, which is classical for these infections [18, 19]. *M. abscessus-chelonae* complex infections were seen in all age groups, in line with these bacteria causing pulmonary infections in patients with cystic fibrosis and other lung diseases, skin and soft tissue infections, catheter-related infections, post-surgical infections, *etc.* [20, 21]. Other species were predominantly seen in patients older than 60 years (*e.g.*, *M. celatum*, *M. simiae* complex, *M. szulgai*, *M. terrae* group and *M. xenopi* group), assumably mostly as a cause of pulmonary NTM infection [20–22]. For some species, age distributions were less characteristic (*M. interjectum*, *M. kansasii*, *M. parascrofulaceum/scrofulaceum* and *M. phocaicum/mucogenicum*), all with more or earlier age peaks, or with more even distributions, possibly reflecting disease in immunocompromised individuals, earlier onset of

TABLE 1 Characteristics of patients with nontuberculous mycobacteria isolated in Denmark from 1991 to 2022 by sex

	Females			Males		
	n	% (95% CI)	IR per 100 000 (95% CI)	n	% (95% CI)	IR per 100 000 (95%CI)
Total	1977	48 (46–49)	2.2 (2.1–2.3)	2146	52 (51–54)	2.5 (2.4–2.6)
Age group in years						
0–14	402	20 (19–22)	2.7 (2.4–2.9)	295	14 (12–15)	1.9 (1.7–2.1)
15–29	86	4.4 (3.5–5.4)	0.5 (0.4–0.6)	138	6.4 (5.4–7.6)	0.8 (0.7–1.0)
30–44	150	7.6 (6.5–8.9)	0.8 (0.7–1.0)	307	14 (13–16)	1.7 (1.5–1.9)
45–59	281	14 (13–16)	1.6 (1.4–1.8)	406	19 (17–21)	2.3 (2.1–2.5)
60–74	601	30 (28–32)	4.4 (4.1–4.8)	641	30 (28–32)	5.1 (4.7–5.5
74+	457	23 (21–25)	5.8 (5.3–6.3)	359	17 (15–18)	7.1 (6.4–7.8)
	n	**% (95% CI)**	**IR per 1 000 000 (95% CI)**	**n**	**% (95%CI)**	**IR per 1 000 000 (95% CI)**
Type of municipality of residence[#]						
Capital	332	17 (15–19)	3.8 (3.4–4.2)	270	13 (11–14)	3.1 (2.8–3.5)
Metropolitan	178	9.0 (7.8–10)	2.0 (1.7–2.3)	153	7.1 (6.1–8.3)	1.8 (1.5–2.1)
Catchment	244	12 (11–14)	2.8 (2.4–3.1)	237	11 (9.8–12)	2.7 (2.4–3.1)
Provincial	335	17 (15–19)	3.8 (3.4–4.2)	333	16 (14–17)	3.8 (3.5–4.3)
Countryside	365	18 (17–20)	4.1 (3.7–4.6)	340	16 (14–17)	3.9 (3.5–4.4)
Unknown	523	26 (25–28)	5.9 (5.4–6.4)	81	38 (36–40)	9.4 (8.7–10.0)
Disease localisation						
Pulmonary	1441	73 (71–75)	16.3 (15.5–17.2)	1410	66 (64–68)	16.2 (15.4–17.1)
Extrapulmonary	492	25 (23–27)	5.6 (5.1–6.1)	614	29 (27–31)	7.1 (6.5–7.7)
Disseminated	44	2.2 (1.6–3.0)	0.5 (0.4–0.7)	122	5.7 (4.8–6.8)	1.4 (1.2–1.7)
Species groups by frequency						
M. avium complex	1306	66 (64–68)	14.8 (14.0–15.6)	1241	58 (56–60)	14.3 (13.5–15.1)
M. abscessus-chelonae complex	133	6.7 (5.7–7.9)	1.5 (1.3–1.8)	142	6.6 (5.6–7.8)	1.6 (1.4–1.9)
M. xenopi group	101	5.1 (4.2–6.2)	1.1 (0.9–1.4)	130	6.1 (5.1–7.2)	1.5 (1.3–1.8)
M. fortuitum–smegmatis group	84	4.2 (3.4–5.3)	1.0 (0.8–1.2)	118	5.5 (4.6–6.6)	1.4 (1.1–1.6)
M. malmoense	75	3.8 (3.0–4.8)	0.8 (0.7–1.1)	93	4.3 (3.5–5.3)	1.0 (0.9–1.3)
M. marinum	36	1.8 (1.3–2.5)	0.4 (0.3–0.6)	92	4.3 (3.5–5.3)	1.1 (0.9–1.3)
M. celatum group	59	3.0 (2.3–3.9)	0.7 (0.5–0.9)	58	2.7 (2.1–3.5)	0.7 (0.5–0.9)
M. kansasii	35	1.8 (1.3–2.5)	0.4 (0.3–0.6)	46	2.1 (1.6–2.9)	0.5 (0.4–0.7)
M. simiae complex	26	1.3 (0.9–1.9)	0.9 (0.2–0.4)	17	0.8 (0.5–1.3)	0.2 (0.1–0.3)
M. parascrofulaceum/scrofulaceum	8	0.4 (0.2–0.8)	0.1 (0.0–0.2)	32	1.5 (1.0–2.1)	0.4 (0.3–0.5)
M. szulgai	8	0.4 (0.2–0.8)	0.1 (0.0–0.2)	31	1.4 (1.0–2.1)	0.4 (0.3–0.5)
M. interjectum	14	0.7 (0.4–1.2)	0.2 (0.1–0.3)	8	0.4 (0.2–0.8)	0.1 (0.0–0.2)
M. terrae group	7	0.4 (0.2–0.8)	0.1 (0.0–0.2)	11	0.5 (0.3–1.0)	0.1 (0.1–0.2)
M. phocaicum/mucogenicum	6	0.3 (0.1–0.7)	0.1 (0.0–0.2)	11	0.5 (0.3–1.0)	0.1 (0.1–0.2)
Other[¶]	79	4.0 (3.2–5.0)	0.9 (0.7–1.1)	116	5.4 (4.5–6.5)	1.3 (1.1–1.6)

[#]: Municipalities of residence were categorised by type of municipality as defined by Statistics Denmark according to the numbers of places of employment and inhabitants in each municipality [14]. [¶]: Other was defined as *Mycobacterium* species and species with n<15 throughout the study period. IR: incidence rate.

pulmonary infection, but also skin and soft tissue infections and catheter-related infections, among others [20–22]. The explanation of high IRs of small children with NTM lymphadenitis, despite being healthy, is relatively unknown. Higher exposure to soil and dust is a plausible mechanism [21]. An immature immune response could also be part of the reason. Children and adolescents comprised most of the extrapulmonary NTM isolation cases (88%, n=636). An increase in NTM lymphadenitis among children has previously been reported [23], but we did not observe this. Interestingly, we saw a seasonal variation with IRs peaking in winter months, which has been reported before [24]. Pulmonary NTM isolation was highly dominant in adults (82%, n=2770). A recent systematic review described risk factors for NTM pulmonary disease and concluded that chronic lung disease is the strongest risk factor [25]. The review also identified studies showing an increasing risk of NTM pulmonary disease with increasing age, while the role of sex was less clear. Over time, accumulation of a potential NTM exposure niche during work, hobbies or living could together with age-related changes in immunity ("immunosenescence") and lung disease, enhance and explain the risk of NTM disease [3, 26].

Several studies have shown NTM infections are more common in females [1, 15–17], while others have shown the opposite [5, 27]. Comparisons between these studies are difficult as the prevalence of smoking and chronic lung disease, and other relevant factors, are not necessarily described. It is speculated that the

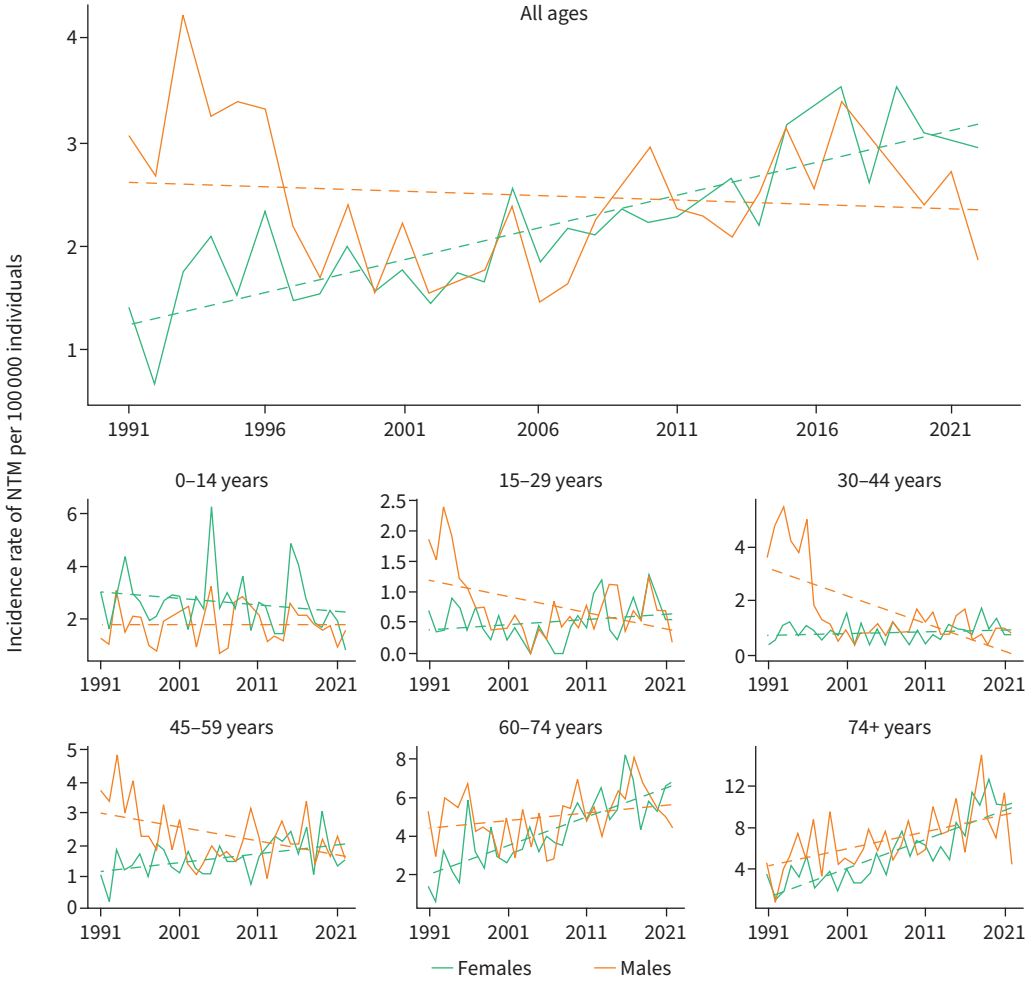

FIGURE 3 Annual incidence rates of nontuberculous mycobacteria (NTM) isolation per 100 000 individuals in Denmark from 1991 to 2022 by age and sex. The black and grey (dashed) lines represent the best-fitted unadjusted linear line through the data.

higher proportion of females among patients with bronchiectasis contributes to this imbalanced sex distribution [25]. A study from Korea with 1017 patients from 2006 to 2016 found that NTM pulmonary disease was more common among females, their peak age was lower than males (59.9 *versus* 66.7 years) and they also had a higher proportion of bronchiectasis (89% *versus* 77%) [1]. The age difference we found was not as prominent. Comparable to our study, an extensive study of more than 16 000 US patients with a diagnostic code of NTM pulmonary disease found an increase in NTM prevalence and incidence among females and older individuals over time [16]. Males generally had higher IRs than females in our study. Still, over time, there were distinct differences in IRs, presumably explained by a change in smoking patterns in the Danish population leading to a relative increase in chronic lung disease among females and a higher risk of NTM [28]. A similar sex shift has also been reported in studies from Australia and New Zealand [29, 30]. Differences in IRs between sexes could be influenced by changes over time in sex distributions in working environments with a higher NTM exposure, but this seems a less obvious explanation than changes in smoking patterns. For example, *M. marinum* infections are more frequent in males, as seen in our study, and are strongly linked with fish-related work and hobbies [31]. However, we cannot think of obvious changes in work environment or exposures in our setting that could explain our findings. Hormonal and genetic factors have also been suggested as part of the sex differences in NTM risk [32, 33]. Healthcare-seeking behaviour could vary between sexes, and over time, although we believe it is unlikely to have changed much during our study period. The decrease in NTM incidence rates among 15–59-year-old males since the early 1990s, especially among 30–44-year-olds, however, is probably explained by the peak of the HIV pandemic and subsequent improvements in prevention and treatment.

TABLE 2 Characteristics of patients with nontuberculous mycobacteria (NTM) isolated in Denmark from 2008 through 2022 by type of municipality of residence

	Type of municipality of residence[#]				
	Capital (n=475)	Metropolitan (n=250)	Catchment (n=403)	Provincial (n=554)	Countryside (n=577)
Age group, years	Incidence rate per 100 000 (95% CI)				
0–14	2.1 (1.7–2.6)	1.9 (1.4–2.7)	2.2 (1.6–2.8)	2.3 (1.9–2.9)	2.2 (1.7–2.8)
15–29	0.6 (0.4–0.9)	0.6 (0.4–0.9)	0.7 (0.4–1.2)	0.6 (0.4–0.9)	0.6 (0.4–1.0)
30–44	0.8 (0.6–1.1)	0.9 (0.6–1.4)	1.1 (0.8–1.6)	1.0 (0.8–1.4)	0.8 (0.6–1.2)
45–59	1.8 (1.4–2.2)	1.8 (1.3–2.5)	1.7 (1.3–2.2)	2.1 (1.7–2.6)	2.1 (1.7–2.6)
60–74	4.3 (3.6–5.1)	5.4 (4.4–6.7)	6.5 (5.6–7.6)	5.6 (4.9–6.5)	6.5 (5.7–7.4)
74+	7.0 (5.8–8.5)	8.2 (6.4–10.6)	7.7 (6.3–9.5)	9.4 (8.0–11.1)	8.9 (7.6–10.4)
Sex					
Females	2.2 (1.9–2.5)	2.4 (2.0–2.8)	3.0 (2.6–3.4)	2.9 (2.5–3.2)	3.4 (3.0–3.8)
Males	1.9 (1.7–2.2)	2.2 (1.8–2.6)	2.9 (2.5–3.3)	2.8 (2.5–3.2)	3.0 (2.7–3.4)
Disease localisation					
Pulmonary	1.5 (1.3–1.6)	1.8 (1.5–2.0)	2.3 (2.1–2.6)	2.2 (2.0–2.4)	2.6 (2.3–2.8)
Extrapulmonary	0.6 (0.5–0.7)	0.5 (0.4–0.6)	0.6 (0.4–0.7)	0.5 (0.4–0.6)	0.6 (0.5–0.7)
Disseminated	0.03 (0.01–0.06)	0.05 (0.03–0.10)	0.04 (0.02–0.09)	0.08 (0.05–0.13)	0.05 (0.03–0.10)
Total	2.1 (1.9–2.3)	2.3 (2.0–2.6)	2.9 (2.6–3.2)	2.8 (2.6–3.1)	3.2 (3.0–3.5)
Species groups by frequency	Incidence rate per 1 000 000 (95%CI)				
M. avium complex	12.0 (10.7–13.6)	13.2 (11.2–15.5)	17.9 (15.8–20.3)	18.4 (16.6–20.4)	21.8 (19.7–24.1)
M. abscessus–chelonae complex	2.3 (1.8–3.0)	2.2 (1.5–3.3)	2.0 (1.3–2.8)	1.5 (1.1–2.2)	1.7 (1.2–2.4)
M. xenopi group	0.6 (0.3–1.0)	2.5 (1.8–4.0)	2.2 (1.6–3.0)	2.4 (1.8–3.0)	1.3 (0.9–2.0)
M. fortuitum-smegmatis group	1.4 (1.0–2.0)	0.9 (0.5–2.0)	1.2 (0.8–2.0)	0.7 (0.4–1.0)	1.1 (0.7–2.0)
M. malmoense	0.4 (0.2–0.8)	0.7 (0.4–1.5)	1.1 (0.7–1.8)	1.1 (0.7–1.7)	1.3 (0.9–1.9)
M. marinum	0.7 (0.4–1.0)	0.9 (0.5–2.0)	0.9 (0.5–2.0)	0.8 (0.5–1.0)	1.3 (0.9–2.0)
M. celatum group	0.7 (0.4–1.0)	0.8 (0.4–2.0)	0.9 (0.5–2.0)	1.1 (0.7–2.0)	1.2 (0.8–2.0)
M. kansasii	0.5 (0.3–0.9)	0.5 (0.2–1.1)	0.6 (0.3–1.2)	0.9 (0.5–1.4)	0.9 (0.5–1.5)
M. simiae complex	0.1 (0.0–0.3)	0.4 (0.1–1.0)	0.3 (0.1–0.8)	0.1 (0.0–0.4)	0.2 (0.1–0.6)
M. parascrofulaceum/scrofulaceum	0.6 (0.3–1.0)	0.1 (0.0–0.6)	0.2 (0.1–0.7)	0.4 (0.2–0.8)	0.1 (0.0–0.4)
M. szulgai	0.3 (0.1–0.6)	0.1 (0.0–0.6)	0.5 (0.2–1.1)	0.2 (0.1–0.5)	0.1 (0.0–0.4)
M. terrae group	0.0 (0.0–0.3)	0.1 (0.0–0.6)	0.1 (0.0–0.6)	0.2 (0.1–0.5)	0.1 (0.0–0.4)

[#]: Municipalities of residence were categorised by type of municipality as defined by Statistics Denmark according to the numbers of places of employment and inhabitants in each municipality [14].

Geographically, there are pronounced differences in NTM species distribution and rates worldwide [3, 6, 7, 34]. Studies have also described within-country differences in NTM rates [5, 15, 16, 27, 35, 36]. We observed that NTM rates varied with age and sex between types of municipalities. Less densely populated municipalities had higher IRs of pulmonary NTM, *M. avium* complex and NTM in older individuals compared with the capital. A US study also found that age-specific rates varied considerably between five states [36]. A study from Croatia has shown that the burden of NTM pulmonary disease is higher in coastal regions compared with the continental region [5]. A spatial epidemiologic analysis from Missouri, USA, found a higher risk of NTM infection was associated with older age, rurality and flooding [35]. In our study, we found a clear tendency of higher NTM IRs associated with decreasing size of municipality of residence (capital to countryside). NTM environmental exposure could be higher in rural areas, but differences in sociodemographic determinants and the overall health of the residents may also bias this finding. After adjusting for age and sex, we still found pronounced differences, which should limit this bias to some degree. Different ethnicities and social disadvantages have also been linked with a higher risk of NTM infection [37, 38], which undoubtedly also varies with geography. As recently described, differences in environmental factors, such as climate, wetlands, precipitation and soil and mineral composition, are potential causes of geographic variations in NTM burden [3]. Finally, mycobacterial testing strategies may vary with hospital setting.

The study was limited by the lack of patient information on disease manifestation (symptomatology and radiological findings), comorbidities and other clinical risk factors for NTM infection. For example, inhaled glucocorticoids and systemic immunosuppression may impact the risk of NTM considerably [39]. Additionally, we did not have access to demographic information such as smoking, income, educational level, work, hobbies and environmental differences (soil and water composition, precipitation, *etc.*), which

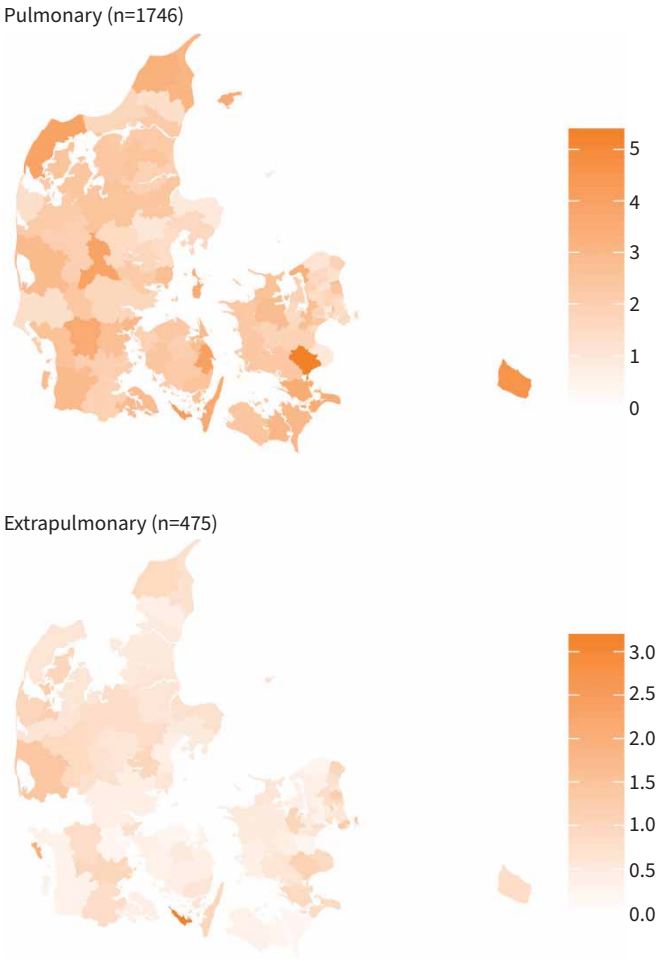

FIGURE 4 Incidence rates of nontuberculous mycobacteria isolation per 100 000 individuals in Denmark from 2008 to 2022 by disease localisation and municipalities in Denmark.

could influence the NTM risk. Still, our large nationwide study provides valuable data on current NTM epidemiology in Denmark over 32 years.

With an evident variation in host susceptibility to NTM infections, understanding differences in age-, sex- and disease-specific immunological and genetic profiles is of considerable clinical interest. The number of

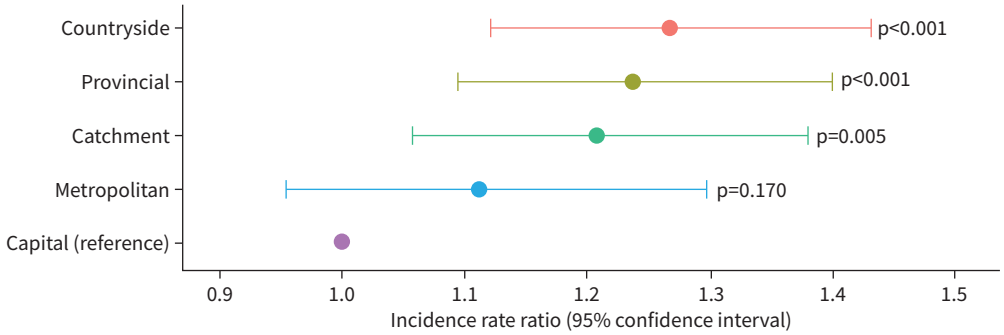

FIGURE 5 Age- and sex-adjusted incidence rate ratios of nontuberculous mycobacteria isolation in Denmark from 2008 through 2022 by type of municipality of residence. Municipalities of residence were categorised by type of municipality as defined by Statistics Denmark according to the numbers of places of employment and inhabitants in each municipality [14].

older patients with NTM pulmonary disease is increasing in our and many other settings, and a higher age is known to be associated with poorer NTM treatment outcomes and more adverse effects [40]. This fact highlights the importance of implementing better treatments and inventing biomarkers that can identify patients at risk of disease and disease progression. Geographic variations in environments undoubtedly contribute to NTM transmission dynamics and a better understanding of this could potentially be useful in clinical practice.

In conclusion, our study demonstrates that simple demographic information, such as age, sex and geography, are essential determinants in NTM epidemiology. We found that pulmonary NTM isolation rates have been increasing over time, particularly in older females, while changes for males were less pronounced. Finally, we observed considerable geographical differences in NTM rates in Denmark, with higher rates in less populated municipalities.

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Data availability: Due to Danish and European law protecting patient privacy, complete data cannot be shared publicly.

Author contributions: V.N. Dahl designed the study with input from A.B. Andersen, T. Lillebaek and C.M. Wejse. A.B. Andersen, T. Lillebaek and C.M. Wejse supervised V.N. Dahl during this work. V.N. Dahl performed all statistical analyses with advice from a biostatistician. V.N. Dahl wrote the first draft. All authors contributed with intellectual content (V.N. Dahl, A.A. Pedersen, J. van Ingen, A.B. Andersen, T. Lillebaek and C.M. Wejse) and provided critical feedback for the revised and final version of the manuscript. All authors read and approved the final version.

Conflicts of interest: V.N. Dahl, A.A. Pedersen, J. van Ingen and A.B. Andersen participate on the advisory board for Nordicinfu Care Denmark, which distributes Arikayce (amikacin liposome inhalation suspension) for Insmed. The other authors declare that they have no competing interests.

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