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Paediatric cervicofacial lymphadenitis caused by non-tuberculous mycobacteria: nation-wide overview in the period 2000–2020

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Abstract

Purpose Cervicofacial lymphadenitis caused by non-tuberculous mycobacteria (NTM) is a rare but increasing infection in children worldwide. The purpose of this study was to analyse and evaluate all microbiologically proven cases of NTM lymphadenitis in children under the age 14 years in Slovenia.

Methods Between 2000 and 2020, we retrospectively reviewed laboratory and medical records for basic demographic and microbiological data. Different clinical samples were collected in medical centres and regional hospitals from all over Slovenia.

Results In the period before mandatory BCG vaccination was discontinued (2000–2005), we did not observe any case of paediatric NTM lymphadenitis. After discontinuation of non-selective BCG vaccination of new-borns (2006–2020), we identified 55 cases of microbiologically confirmed NTM lymphadenitis in BCG-unvaccinated children with median age 26.0 months (range: 15.0–75.0 months). Mean annual incidence of paediatric NTM lymphadenitis accounted for 1.26 (range: 0.35–2.38) per 100,000 children. The main causative agents were *Mycobacterium avium* (38/55; 69.1%) and *M. intracellulare* (9/55; 16.4%). We did not find any *M. chimaera* isolate. Since 2006, each year we microbiologically confirm sporadic cases of paediatric NTM lymphadenitis, a condition not diagnosed before.

Conclusions After discontinuation of universal BCG vaccination in March 2005, first cases of paediatric NTM lymphadenitis appeared. Several possible reasons could be attributed to observed trend and further multinational observational studies are warranted to explore possible causal relationships.

Keywords Cervicofacial lymphadenitis, Paediatric lymphadenitis, Non-tuberculous mycobacteria, *Mycobacterium avium*, *Mycobacterium intracellulare*, Mycobacterial lymphadenitis

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Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous saprophytes found in different ecological niches in aquatic and terrestrial environments [1]. To date, genus *Mycobacterium* includes over 190 different mycobacterial species [2, 3] with some of NTM species representing as opportunistic pathogens associated with pulmonary, soft tissue, lymphadenitis and/or disseminated diseases in humans [4]. According to published literature, infections and diseases caused by NTM are increasing worldwide and several possible reasons (e.g.: aging of human population, widespread use of immunosuppressive therapy, increase focus on NTM infections etc.) have been proposed to explain this global trend [4–9].

In children, the clinical presentation of an NTM infection typically consist of a subacute or chronic unilateral lymphadenopathy in the anterior cervicofacial triangle (cervicofacial lymphadenitis) often followed by spontaneous drainage and fistula. Spontaneous regression can occur in a significant proportion of cases. Early diagnosis of NTM lymphadenitis is difficult as most lesions are generally associated with minimal or negligible tenderness and heating sense. Patients with NTM lymphadenitis rarely experience systemic symptoms such as fever, weight loss, malaise, and night sweats. After several weeks in disease course, patients may show discoloration, with thinning, peeling, and scaling of the overlying skin [10, 11]. Cervicofacial NTM lymphadenitis is an illness affecting predominantly children up to the age of 5 years [10, 12–14], although cases of lymphadenitis in elder children and adolescents have also been reported [11]. Most cases of cervicofacial NTM lymphadenitis still occur in otherwise healthy, immunocompetent children who are not reported to be prone to opportunistic infections later in life [15–17]. The infectious agent usually enters through the orofacial area through the consumption of infected water or food. Why the infection occurs in the very young ones remains unknown, but it may be related to the growth of primary teeth, use of mouth as explorative instrument, and intensive exposure to environmental mycobacteria in playing grounds [15]. Declining rates of tuberculosis (TB) in most industrial countries have left NTM as the most common cause of mycobacterial cervicofacial lymphadenitis in young children (age under 5 years). Some retrospective [14, 18–20] and prospective [12, 13, 21] studies have focused in the incidence, characteristics and possible risk factors of NTM lymphadenitis in children.

Many NTM species have been linked with subacute lymphadenitis in the head and neck region in children. It seems that distribution and frequency of mycobacterial species involved in paediatric lymphadenitis is dependent on the geographical location, but the main causative agent is *M. avium-intracellulare* complex followed by *M. scrofulaceum*, *M. lentiflavum*, *M. fortuitum*, *M. haemophilum* and others [12, 18, 20, 22, 23].

The diagnosis of cervicofacial lymphadenitis is usually based on cytological, histological and/or microbiological examination of lymph node specimens from patients who fail to respond to the standard antibiotic treatment for bacterial lymphadenitis. In microbiology, isolation of NTM in culture still represents the most reliable and the most sensitive method for detection of NTM as causative agents of paediatric lymphadenitis. In last few years, methods of molecular biology offer rapid and simultaneous detection of NTM species directly in clinical specimen or in culture. However, their performance and reliability is still limited [10, 15, 24].

The recommended treatment is complete excision of the affected lymph node, with a success rate over 90% [10, 15, 24]. Due to risk of serious adverse events (e.g. facial nerve palsy, wound infection, scar formation, fistula formation) surgery is not always appropriate. In those cases, antibiotics are regarded as a sole or adjuvant therapy [16, 25]. Despite good initial cure rate, subsequent treatment courses are necessary in substantial number of patients and recurrences can be observed up to 5 years later [25]. It has also been reported that observation alone is a good alternative to surgery, without the risk of complications [26, 27]. Taken all together, it is obvious that treatment of paediatric NTM lymphadenitis is not standardized. It requires personalized approach with careful choice of intervention and active follow-up.

Slovenia is a small, independent Central European country with approximately 2 million inhabitants. The laboratory diagnosis of mycobacteria is centralised into two diagnostic laboratories, Laboratory for Mycobacteria Golnik (University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia) and Laboratory for Mycobacteria Maribor. Laboratory for Mycobacteria Golnik performs all the procedures on primary and national level, while Laboratory for Mycobacteria Maribor performs diagnosis of mycobacteria at primary level and sends all positive cultures for identification and drug susceptibility testing to our laboratory. Due to low national incidence of TB cases and the partial protective effect of the BCG vaccine against TB, mandatory nation-wide BCG vaccination of new-borns was discontinued in March 2005. After that date, we use selective BCG vaccination of new-borns of immigrant families who moved to Slovenia from countries with a higher incidence of tuberculosis [28].

The aim of our study was to analyse and evaluate all microbiologically proven cases of paediatric NTM lymphadenitis in the period 2000–2020 in Slovenia. This retrospective study presents the distribution of NTM isolates causing lymphadenitis in children after 2005 when non-selective mandatory BCG vaccination of new-borns was discontinued and shows annual incidence rate of

paediatric NTM lymphadenitis in our country. To our knowledge, this is the first study reporting paediatric NTM lymphadenitis in the region of Balkan Peninsula.

Methods

Inclusion of patients, collection of demographic and Microbiological data

Between 2000 and 2020, we included all children under the age 14 years with microbiologically proven NTM cervicofacial lymphadenitis. All children included had persistent enlargement of lymph nodes in head or neck area. All microbiological cultures were negative for other causative agents. For that reason, testing for mycobacteria was indicated.

We retrospectively reviewed medical and laboratory records and data obtained from Laboratory for Mycobacteria and National Registry for Tuberculosis Republic of Slovenia (both located at University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia) for basic demographic data, information regarding chronic illnesses and microbiological data. In our country, NTM infections are not included in mandatory reporting, and thus, the burden of NTM disease is unknown. For that reason, we also reviewed the same data to assess the distribution of all NTM species identified in Laboratory for Mycobacteria Golnik.

The incidence of paediatric NTM lymphadenitis was calculated using population age-distribution data from the Statistical Office of Republic of Slovenia (Ljubljana).

Laboratory diagnosis For definitive microbiological diagnosis of NTM cervicofacial lymphadenitis in affected children, different clinical samples (biopsy, aspiration or excision of affected lymph node, mycobacterial isolates) were obtained and sent to Laboratory for Mycobacteria Maribor or Golnik. Clinical samples were collected in medical centres and regional hospitals from all over Slovenia. From one patient several different clinical samples could be obtained. Briefly, clinical materials obtained from non-sterile anatomical sites were decontaminated with NALC-NaOH decontamination, a protocol that is routinely used in Laboratory for Mycobacteria. Clinical materials obtained from sterile sites were not decontaminated. Auramine stain was performed on specimens to detect acid-fast bacilli in smears examined microscopically. The rest of the specimen was inoculated in liquid mycobacteria growth indicator tube medium (BACTEC MGIT 960, BD Diagnostic Systems, Franklin Lakes, NJ, USA) and on two slopes of solid media, Lowenstein-Jensen (LJ; Remel, San Diego, CA, USA) and Stonebrink (ST). All media were incubated at 36 °C (± 1 °C) and additional MGIT at 29 °C (± 1 °C). For possible isolation of *M. haemophilum*, all specimens were additionally inoculated on LJ medium with added iron citrate and incubated at 29 °C (± 1 °C). Moreover, if requested by clinicians, molecular, PCR-based test GenoType CMdirect (Hain, Nehren, Germany) was performed directly from clinical specimens.

In case of mycobacterial growth, NTM species were identified in Laboratory for Mycobacteria Golnik using GenoType CM, AS and/or NTM-DR molecular tests (Hain, Nehren, Germany). All mycobacterial isolates identified before 2015 as *M. intracellulare* were retested with GenoType NTM-DR test to distinguish between *M. intacellulare* and *M. chimaera*.

Statistical analysis was performed in GraphPad v. 6.04.

Results

Demographic characteristics of children with microbiologically proven lymphadenitis

In the period 2000–2005, we did not observe any case of paediatric lymphadenitis caused by NTM (Table 1; Fig. 1). First case of NTM lymphadenitis was identified at the end of 2006, a year and a half after nonselective BCG vaccination was discontinued. Interestingly enough, even other NTM infections were rarely observed in children in the period 2000–2005 (Fig. 1).

Between 2006 and 2020, we identified 55 children with microbiologically proven cervicofacial NTM lymphadenitis. Among the children included, no NTM infections of lung, skin, or bones were observed. There was one case of atopic dermatitis, one case of asthma and one case of iron deficiency anaemia.

Basic demographic characteristics of children included in this study are presented in Table 2. Median age at symptom onset was 26.0 months (range 15.0–75.0 months), with no statistical difference between male vs. female gender (24.5 months vs. 28.0 months, $p = 0.923$; unpaired t test). The majority of patients with cervicofacial lymphadenitis (24/55; 43.6%) belonged to the age group 13–24 months. All included children have Slovene background and have not been vaccinated with BCG vaccine. In the period 2006–2020, mean annual incidence of NTM lymphadenitis in children younger than 14 years accounted for 1.26 (range: 0.35–2.38) per 100,000 children (Table 1; Fig. 1).

Distribution of non-tuberculous mycobacteria as causative agents of child lymphadenitis between 2000 and 2020

Table 1 represent the number of cases of cervicofacial lymphadenitis in children per year in the period 2000–2020. A quarter of paediatric NTM cases were smear positive (14/55; 25.5%; Table 3). Approximately one third of samples were also tested with PCR-based method GenoType CM*direct* (15/55; 27.3%). Of those, only four (4/55; 7.3%) were positive for mycobacteria. Three of them were PCR positive for *M. avium* and confirmed with positive culture for *M. avium.* In one case, GenoType CM*direct*

Table 1 Distribution of non-tuberculous mycobacteria species as causative agents of cervicofacial lymphadenitis in children and corresponding annual incidence rates in Slovenia between 2000 and 2020

Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total (%)
No. of NTM cervicofacial lymphadenitits*	0	0	0	0	0	0	2	1	2	1	2	5	7	5	5	2	3	5	4	2	9	55 (100)
Non-tuberculous mycobacteria species																						
M. avium							2	1	1		2	4	6	3	3	2	2	3	2	1	6	38 (69.1)
M. intracellulare									1	1		1		1				2	1	1	1	9 (16.4)
M. kansasii														1							2	3 (5.5)
M. lentiflavum															1				1			2 (3.6)
M. interjectum													1									1 (1.8)
M. celatum															1							1 (1.8)
M. scrofulaceum																	1					1 (1.8)
Incidence rate (per 100,000 children)	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.36	0.71	0.35	0.70	1.72	2.40	1.68	1.66	0.66	0.98	1.62	1.93	0.63	2.83	1.26

Footnote: *Only microbiologically confirmed cases of NTM cervicofacial lymphadenitis in children were included in this study. In Slovenia, BCG vaccination was discontinued in March 2005

was positive for *Mycobacterium* species, while in culture *M. intracellulare* was detected.

The main causative agent of child lymphadenitis was *M. avium* (38/55; 69.1%), followed by *M. intracellulare* (9/55; 16.4%), *M. kansasii* (3/55; 5.5%) and sporadic cases of *M. lentiflavum*, *M. interjectum*, *M. celatum* and *M. scrofulaceum* (Table 1). The first two microbiologically proven cases of child lymphadenitis, both caused by *M. avium*, appeared in November and December 2006. For differentiation between *M. intacellulare* and *M. chimaera*, all isolates of *M. intarcellulare* were retested with GenoType NTM-DR test, which was commercially available in 2016, and all of them proved to be *M. intracellulare*.

Frequency of non-tuberculous mycobacterial species in general population in Slovenia between 2000 and 2020

Table 4 presents the number and percentage of NTM species found in cases of child lymphadenitis identified from any human clinical samples in Slovenia between 2000 and 2020. In that period, 6225 mycobacterial species were isolated and identified. The first and the second most commonly identified mycobacterial species in Slovenia were *M. xenopi* (1718/6225; 27.60%) and *M. gordonae* (1115/6225; 17.91%) respectively (data not presented in the table). Both, *M. xenopi* and *M. gordonae*, were not identified in neither case of NTM lymphadenitis. *M. avium*, which is the most common cause of child lymphadenitis, was identified in general population in 15.33% (954/6225) of clinical specimens and was the third most commonly identified mycobacterial species in our country in 21-years long period.

Discussion

In last two decades, we observe accelerated decline in TB incidence in Slovenia. In 2000, TB incidence dropped for the first time below 20 per 100,000 population and it is declining ever since; in 2020 it accounted for only 3.7 per 100,000 population [29]. On the other side, we can observe just the opposite trend for diseases caused by NTM species. The number of those is slowly rising. In 2015, the number of NTM isolates have outnumbered for the first time the *Mycobacterium tuberculosis* isolates among Slovenian patients [30, 31]. To our knowledge, this is the first study reporting cervicofacial NTM lymphadenitis in children in Balkan Peninsula. In the frame of our study, we focused on microbiologically confirmed cases and it is likely that NTM lymphadenitis in children is under-reported. Spontaneous resolution of cervicofacial lymphadenitis has been previously reported [12, 26] and therefore, at least these cases were most likely missed in the frame of our study.

In Slovenia, after discontinuation of mandatory BCG vaccination in March 2005, we observe between one to nine cases of paediatric NTM lymphadenitis each year.

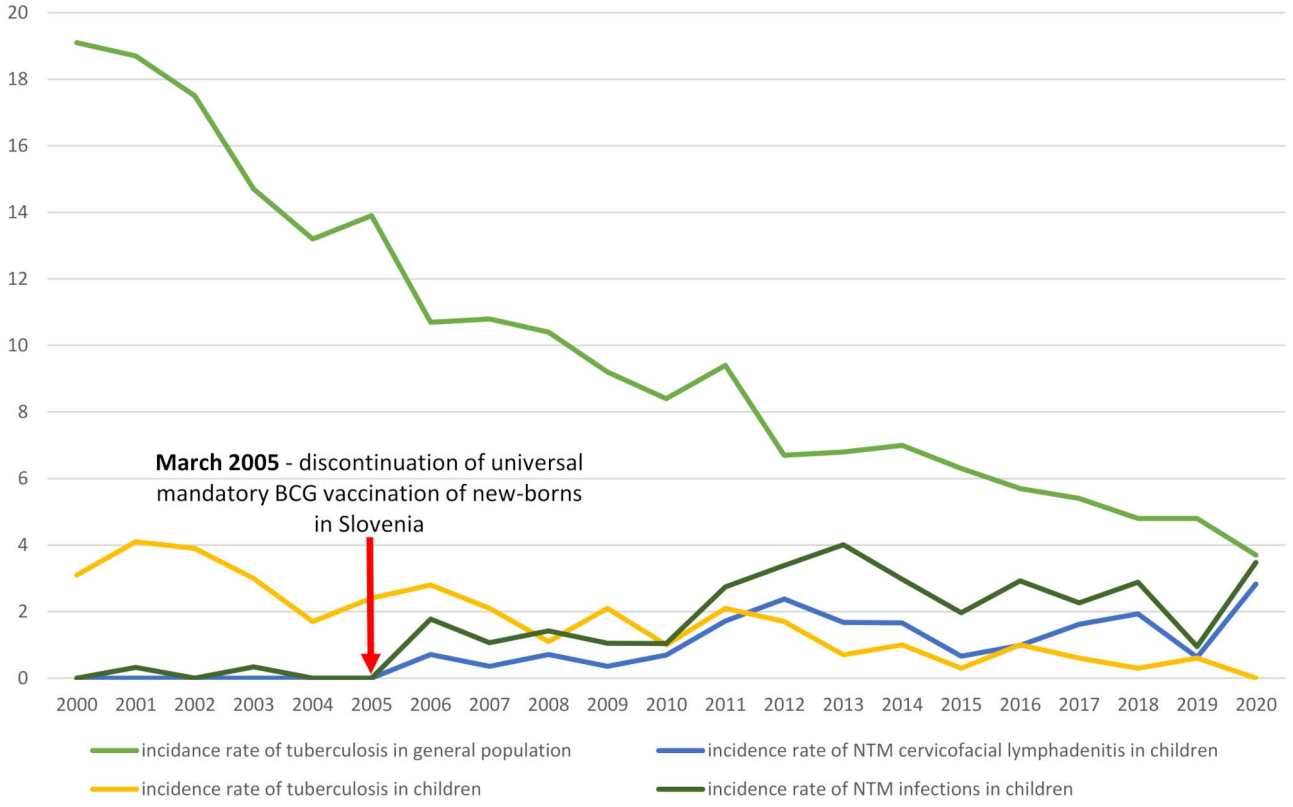

Fig. 1 Annual incidence rates of tuberculosis in general population, tuberculosis in children, non-tuberculous (NTM) cervicofacial lymphadenitis in children, and NTM infections in children in Slovenia between 2000 and 2020

Footnote: Annual incidence rates of tuberculosis in general population were retrieved from the report of National Registry for Tuberculosis Republic of Slovenia and Laboratory for Mycobacteria (University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia). Children younger than 14 years were included to calculate annual incidence rates of tuberculosis, NTM cervicofacial lymphadenitis and NTM infections

Table 2 General demographic characteristics of children with NTM cervicofacial lymphadenitis included in this study

Characteristic	Number (%)
All patients	55 (100)
Gender	
female	31 (56.4)
male	24 (43.6)
Age (median, range)	26.0 months (15.0–75.0)
Age (months)	
0–12	0 (0.0)
13–24	24 (43.6)
25–36	15 (27.3)
37–48	12 (21.8)
49–60	0 (0.0)
61–72	3 (5.5)
73–84	1 (1.8)

Observed mean annual incidence accounts for 1.26 (range: 0.35–2.38) per 100,000 children. Before that period, we did not detect any case of microbiologically proven paediatric NTM lymphadenitis. This observation is consistent with the published literature that recognise NTM cervicofacial lymphadenitis as a rare but increasing infection in children. Estimates of the annual incidence of NTM lymphadenitis in children range from 0.53 to 5.7 per 100,000 children [13, 18–21, 32]. For example, Reuss et al. [13] estimated a cumulative incidence rate for children younger than 15 years of 3.3/100,000 with incidence rate of children younger than 4 years of 11.3/100,000. Moreover, some studies compared the incidence of cervicofacial lymphadenitis caused by NTM before and after the discontinuation of widespread BCG vaccination. All these studies came to the same conclusion and that is increase in incidence of paediatric lymphadenitis caused by discontinuation of BCG vaccination [14, 18–20, 32]. However, we must take into consideration that published articles vary greatly in study designs and/or how the confirmation of lymphadenitis was done. In several studies, cases with microbiologically confirmed NTM lymphadenitis were included while other published studies included lymphadenitis cases with diagnosis based on a single positive diagnostic test result, on a combination of findings, or even on clinical presentation only. This might influence the reported incidence rates of paediatric lymphadenitis.

Table 3 Number and percentage of paediatric NTM lymphadenitis cases according to Microbiological detection method

Microbiological detection method	Auramin smear *N* (%)	PCR-based GenoType CMdirect *N* (%)	Culture positive for NTM** *N* (%)
	Total cases of paediatric NTM lymphadenitis (*N* = 55)		
positive	14 (25.5)	4 (7.3)	55 (100.0)
negative	41 (74.5)	11 (20.0)	0 (0.0)
ND	0 (0.0)	40 (72.3)	0 (0.0)

Footnote: ND– not done; NTM– non-tuberculous mycobacteria *PCR-based test GenoType CM*direct* was performed directly from patient specimen upon clinicians' request, **culture-based methods still represent gold standard for detection of NTM

BCG vaccination is widely utilized worldwide to protect against infection with tubercle bacilli. Its goal is to establish a stable population of long-lived memory T cells. Several studies proposed that BCG vaccination protects against early post-vaccination exposure to *M. tuberculosis*, but is ineffective with later exposure [33]. For that reason, several European countries discontinued mandatory BCG vaccination, among them also Slovenia. Main reasons for this particular decision in Slovenia were rapid decrease of national incidence of TB cases (13.2 per 100,000 population in 2004), low incidence and low number of deaths due to tuberculosis meningitis [34]. Discontinuation of BCG vaccination in several countries worldwide has been recognized as one of the main reasons for increasing incidence of NTM lymphadenitis in children. Some other studies reported statistically significant increase of incidence rate of paediatric NTM lymphadenitis after the discontinuing of BCG vaccination [14, 18–20, 32]. This is concordant with the results of recently published meta-analysis [6], which observed that incidence of NTM lymphadenitis is greatly reduced among BCG-vaccinated children compared with BCG-unvaccinated children. The authors of this study [6] concluded that protective effect of BCG vaccination against NTM should be taken into consideration when deciding on recommendations for discontinuation of universal BCG vaccination programs and in assessing new vaccines designed to replace BCG. Slovenia is the first country from Balkan Peninsula that discontinued mandatory BCG vaccination for all new-borns. All other countries in that region still hold mandatory national BCG vaccination policy [28].

According to the published literature, the protective effect of the BCG vaccine on NTM lymphadenitis is considered as very probable in the long term due to cross immune response. Epidemiological studies indicate that BCG vaccination is associated with marked decrease in *M. avium* disease prevalence [35]. Similarly, latent TB infection decreases the risk of NTM disease further indicating the importance of cross-protective immunity [36]. Moreover, Abate et al. [37] performed several experiments on peripheral blood mononuclear cells obtained from the blood of healthy volunteers and observed that BCG vaccination of healthy volunteers induces TB and NTM cross-reactive T cells.

Most of the cases of paediatric NTM lymphadenitis in the present study were confirmed by culture-based methods, which still represent gold standard for NTM detection. One might contribute increased incidence of paediatric NTM lymphadenitis to improvements in diagnostic procedures. Since nothing has changed considerably in culture methods in our laboratory over the mentioned timeframe, this might play only minor role. One of the possible reasons could be improved awareness (also in surgeons and physicians involved in clinical assessment) in recognizing NTM as causative agents of NTM lymphadenitis. The same reason possibly applies to other NTM infections as NTM were rarely recognized causative agents in children in the period 2000–2005.

In developed countries, *M. avium* is the most common mycobacterial species causing paediatric NTM lymphadenitis [13, 14, 17–20, 23, 32] ranging from 32.3% [23] to 83.3% [13]. That observation is concordant with the results of our study. *M.avium* was detected in majority of the cases (38/55; 69.1%).

Interestingly enough, after retesting all *M. intracellulare* isolates with molecular test GenoType NTM-DR, which distinguish between *M. intracellulare* and *M. chimaera*, we did not identify any *M. chimaera* isolate. This observation might indicate that *M. chimaera* is less virulent compared to *M. intracellulare* as suggested in our previous study [30]. Moreover, in the general population *M. chimaera* could be isolated from clinical samples in similar proportion as *M. intracellulare.* We did not assess clinical importance of these isolates in the frame of the presenting study but this finding indicate that other factors (immunogenic factors, host-pathogen interactions, routes of transmission etc.) might explain why we could not find any *M. chimaera* isolate in paediatric NTM lymphadenitis. Furthermore, data on geographical/environmental distribution and frequency of *M. chimaera* are limited since most of the published literature did not differentiate between *M. intracellulare* and *M. chimaera*.

According to some reports, *M. haemophilum* seems to be frequent cause of paediatric lymphadenitis [22–24]. Even more, according to study conducted by Dutch authors, *M. haemophilum* was the second most common cause of paediatric lymphadenitis [22]. This observation was confirmed by the results of meta-analysis, which included 1951 children from sixty publications

Table 4 Frequency of non-tuberculous mycobacterial species in general population in Slovenia between 2000 and 2020

Mycobacterium (M.) sp.	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total 2000–2020
All	180	182	170	213	136	211	207	182	178	299	282	321	316	401	384	418	495	437	374	522	317	6225
M. avium	18	13	18	38	7	27	41	39	38	52	42	42	59	63	51	69	63	78	56	85	55	954
%	10.00	7.14	10.59	17.84	5.15	12.80	19.81	21.43	21.35	17.39	14.89	13.08	18.67	15.71	13.28	16.51	12.73	17.85	14.97	16.28	17.35	15.33
M. intracellulare	5	12	10	21	12	19	10	17	16	22	12	18	18	30	27	26	17	21	21	34	21	389
%	2.78	6.59	5.88	9.86	8.82	9.00	4.83	9.34	8.99	7.36	4.26	5.61	5.70	7.48	7.03	6.22	3.43	4.81	5.61	6.51	6.62	6.25
M. chimaera	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	17	15	28	20	2	82
%																	3.43	3.43	7.49	3.83	0.63	1.32
M. kansasii	11	9	16	16	5	16	26	23	14	27	18	36	18	28	29	15	33	40	19	23	14	436
%	6.11	4.95	9.41	7.51	3.68	7.58	12.56	12.64	7.87	9.03	6.38	11.21	5.70	6.98	7.55	3.59	6.67	9.15	5.08	4.41	4.42	7.00
M. lentiflavum	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	2	2	0	8
%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.26	0.24	0.00	0.23	0.53	0.38	0.00	0.13
M. interjectum	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	1	3	0	2	2	2	14
%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.78	0.24	0.61	0.00	0.53	0.38	0.63	0.22
M. celatum	0	0	2	2	0	1	2	2	5	2	5	0	6	6	5	2	10	7	12	8	2	79
%	0.00	0.00	1.18	0.94	0.00	0.47	0.97	1.10	2.81	0.67	1.77	0.00	1.90	1.50	1.30	0.48	2.02	1.60	3.21	1.53	0.63	1.27
M. scrofulaceum	2	7	0	0	0	0	0	1	0	2	0	1	1	1	4	2	2	1	1	6	3	34
%	1.11	3.85	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.67	0.00	0.31	0.32	0.25	1.04	0.48	0.40	0.23	0.27	1.15	0.95	0.55
Other mycobacteria*	144	141	124	136	112	148	128	100	105	194	205	224	213	272	264	302	350	274	233	342	218	4229
%	80.00	77.47	72.94	63.85	82.35	70.14	61.84	54.95	58.99	64.88	72.70	69.78	67.41	67.83	68.75	72.25	70.70	62.70	62.30	65.52	68.77	67.94

Footnote: *Other mycobacteria includes all other mycobacteria identified (but not found in paediatric NTM lymphadenitis with the exception of *M. chimaera* and therefore not presented in this table) in Laboratory for Mycobacteria (University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia). In this category are also some of the most commonly identified mycobacteria in Slovenia: *M. xenopi* (as the first most commonly isolated mycobacteria), *M. gordonae* (as the second), *M. fortuitum group*, and others. ND = not detected with available molecular tests used in laboratory at that time

[24]. Distinct from most other NTM, *M. haemophilum* is unable to produce iron-binding siderophores, therefore it requires iron supplementation to grow in culture. Due to its fastidious growth requirements, *M. haemophilum* may be underdiagnosed and more prevalent than previously reported. In the frame of our study, we did not identify any case of paediatric lymphadenitis caused by *M. haemophilum*, although we used media with iron supplement to boost its growth. Some of the reasons are probably its geographic distribution and the small number of NTM lymphadenitis cases included in our study. Cases of all diseases caused by *M. haemophilum*, including paediatric lymphadenitis, have been described in some parts in the world, including France, Italy, Germany, the Netherlands, the United Kingdom, Israel, parts of Africa, Australia, Canada, the US and Brazil [22–24, 38, 39]. The importance of its geographical source in paediatric lymphadenitis was also confirmed by meta-analysis, which pointed out that all the cases of *M. haemophilum* lymphadenitis originated from two countries only, the Netherlands and Israel [24]. Besides that, it seems that *M. haemophilum* affects children older than 4 years (48 months) [11, 22]. The percent of those children included in our study was very low (children over 48 months: 4/55; 7.3%). Interesting enough, study from the Netherlands pointed out that *M. haemophilum* lymphadenitis was more common in children with non-Dutch background [22]. Furthermore, in multivariate analysis conducted in that particular study [22], only older age and a non-Dutch background were predisposing risk factors for *M. haemophilum* infection, compared with *M. avium* infection. In our study, all children with NTM lymphadenitis included have Slovene background.

Study limitations

Slovenia has only two diagnostic laboratories in which identification of mycobacteria is performed thus all microbiologically proven cases of paediatric NTM lymphadenitis in our country were included in this study.

However, some cases of NTM lymphadenitis were most likely missed due to several possible reasons. Spontaneous resolution of cervicofacial lymphadenitis has been previously reported [12, 26] and consequently, at least these cases were most likely missed in the frame of our study. One possible reason for missed cases of paediatric NTM lymphadenitis might also be weaker awareness of medical staff included in its clinical assessment. Therefore, there is a possibility that samples for mycobacterial cultivation were not collected and not examined for mycobacteria in any of the two laboratories in Slovenia. Awareness increased in the past few years and more cases of not only paediatric NTM lymphadenitis but also other NTM infections could be discovered. Moreover, in cases of high clinical suspicion based on history and examination only, patients may sometimes receive diagnosis of probable NTM lymphadenitis and undergo surgical intervention without a confirmed diagnosis with PCR or culture [40, 41]. Such cases were not subject of our study.

Another possible reason could also be false negative result of microbiological examination. Recently published systematic review [41] investigated the sensitivity of various diagnostic methods used in the work-up of paediatric NTM lymphadenitis. The authors of that large study detected 10 different studies (with 494 patients included all together) that reported the sensitivity of NTM culture. Culture as diagnostic method in the assessment of paediatric NTM lymphadenitis showed a sensitivity of only 0.54 (95% CI 0.50 to 0.58).

Another limitation of our study is its retrospective and descriptive design. We simply reported the annual incidence of paediatric NTM lymphadenitis that was microbiologically proven in the period 2000–2020 and discussed possible reasons for the observed increasing incidence. Descriptive nature of our research did not allow us to estimate controlled association between discontinuation of BCG vaccination or any other risk factor and increased incidence of NTM lymphadenitis in children.

Conclusions

Although NTM lymphadenitis seems to be a rare condition mainly diagnosed in younger children, its incidence is increasing not only in Slovenia but also worldwide. Several possible reasons could be attributed to observed increasing incidence and further multinational observational studies are warranted to explore possible causal relationships. It might be interesting to study incidence of paediatric cervicofacial lymphadenitis caused by NTM in countries where BCG vaccination is still mandatory.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Eva Sodja, Urška Šivic and Manca Žolnir Dovč. The first draft of the manuscript was written by Eva Sodja and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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None to declare.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This retrospective observational study was approved by the Slovenian National Medical Ethics Committee (approval number 0120 – 94/2021/3)

and was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki. The requirement for informed consent was waived owing to the retrospective observational nature of the study. The decision not to require informed consent was upheld by the Slovenian National Medical Ethics Committee. The research does not pose additional burdens for children included in this study and does not reveal their personal data.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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