Journal of Infection and Public Health 12 (2019) 803–808

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Burden of non-tuberculous mycobacterial diseases in Saudi Arabian children: The first nationwide experience

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ARTICLE INFO

Article history:
Received 7 February 2019
Received in revised form 19 March 2019
Accepted 2 April 2019

Keywords:
Pediatric non tuberculous mycobacterial disease
Saudi Arabia
NTM epidemiology
Mycobacterium simiae
M. riyadhense
M. abscessus
M. kubicae
M. monacense

ABSTRACT

Background: Non-tuberculous mycobacteria (NTM) causing pulmonary and extra-pulmonary diseases are increasing worldwide. A large paucity of data related to pediatric NTM diseases exists globally and particularly in Saudi Arabia.
Methods: The first nationwide exploratory study on existence of NTM diseases among Saudi Arabian children (0–14 years old) has been carried out during 2016–2017. Suspected NTM isolates with clinical and demographical data were enrolled from regional reference laboratories. Species level identification of isolates was carried out by commercial line probe assays and gene sequencing.
Results: In 12 months, 52 culture positive cases with 44(84.6%) confirmed disease incidences were identified. Demographically, Saudi nationals (86.5%) were dominated and 77.3% cases have different comorbid conditions. Lymphadenitis (40.4%) followed by 26.9% of pulmonary cases with 42.8% of confirmed clinical relevance were mainly reported. Species identification showed *Mycobacterium simiae* (31.8%), *M. abscessus* (23.1%) and nine other species including rarely encountering *M. riyadhense*. Ascites caused by *M. monacense*, pulmonary disease caused by *M. riyadhense* and *M. monacense* were rarest clinical events and reported for the first time globally in a pediatric cohort.
Conclusions: Diverse NTM diseases even in immunocompetent children are an upcoming challenge in Saudi Arabia. Lack of awareness on NTM disease must be addressed with immediate development of management plans.

Introduction

Non-tuberculous mycobacteria (NTM) are opportunistic emerging pathogens causing broad spectrum of pulmonary and extra-pulmonary infections in humans. To date, more than 150 species of NTM's are recognized and more than 50 species among them are defined as human pathogens [1]. Different species of NTM's were proved to be etiological agents of causing pneumonia, lymphadenitis, bacteremia, disseminated infections, infection to skin, soft tissues, eyes, bone and joints, central nervous system, gastrointestinal system and genitourinary systems. In the last decade, a significant increase in NTM disease across the globe has been noticed even among immunocompetent individuals, although the right reason behind the rise is unclear [2]. Despite the developments in diagnosis and management of NTM associated diseases, a paucity of data exist on pediatric NTM diseases. In addition, the American Thoracic Society (ATS) highlighted in their guidelines the existing uncertainty in the optimal management of NTM associated infections in pediatric groups [3].

Following the global trend, Saudi Arabia also has inadequate information on pediatric NTM disease spectrum and epidemiology. To date, consolidated data on nationwide burden of NTM diseases among pediatric patient groups is unavailable although some recent studies reported increasing prevalence of vari-

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https://doi.org/10.1016/j.jiph.2019.04.004

ous NTM species in Saudi Arabia [4–6]. However, few case reports from different regions of the country demonstrated the pathogenic potential of NTM's among Saudi Arabian children [7–9]. Immunosuppressive conditions including genetic disorders were considered as the most common underlying conditions behind NTM infections in adults as well as in children [10,11]. In Saudi Arabia, research on possible risk factors including genetic disorders among pediatric NTM disease cohorts are not yet available.

To characterize the current existence of pediatric NTM diseases in the country, a nationwide prospective exploratory study has been carried out for a period of 12 months with culture positive NTM cases belonging to the age group 0–14 years. For the first time in the country, the spectrum of NTM diseases was analyzed with comprehensive collection of clinical and demographic data.

Materials and methods

Study design

During September 2016–August 2017, from regional reference laboratories located in Riyadh, Dammam, Jeddah, Jizan, Hail, Madina, AlBaha, Taif, Jizan and Aseer, NTM isolates reported from children aged below 14 years were collected. These laboratories are reporting facilities of mycobacterial cases under national TB control programs and they adequately represent the population of each province. This study is an exploratory nationwide analysis, therefore direct patient recruitment or follow-up was not under the scope. We included cases reported from pediatric category with at least one positive culture. In addition, during 12 months period, if a case was reported with multiple positive culture, all the isolates were enrolled. A standard data collection form has been used to extract clinical and demographic data from available laboratory records. The data collection was strictly anonymous and patient identifiers were omitted throughout collection and analysis period. All the collected isolates were sub-cultured into LJ medium and transferred from national study sites to Mycobacteriology research facility in Riyadh along with the data collection form.

The enrolled isolates were classified into two major case groups namely pulmonary and extra-pulmonary based on anatomical site of infection. Pulmonary isolates were acquired from sputum, bronchio-alveolar lavage, gastric aspirates and tracheal aspirate samples. We followed the American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) guidelines for the diagnosis of NTM diseases [3]. The clinical relevance of each pulmonary cases were confirmed by following the ATS/IDSA guidelines. Briefly, a single isolation from sputum or tracheal tube aspirate was considered as NTM colonization and not clinically significant. On the other hand, cases which reported with appropriate clinical, radiological findings along with repeated culture positivity (minimum two different occasions) only were considered as clinically relevant. Isolates from any sites of infection other than pulmonary was defined as the extra-pulmonary group, which includes sterile (ex; body fluids) and non-sterile body sites (ex; skin).

The study has been reviewed and approved by Office of Research Affairs at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Identification of NTM species

All the suspected isolates received from referral laboratories were sub-cultured into both liquid (MGIT, Becton Dickinson, USA) and solid media (Lowenstein-Jensen's slants). Genomic DNA was extracted from all isolates by using commercially available spin column technique (Qiagen, Germany). All the DNA samples were subjected to primary species identification by using commercially available line probe assays- Genotype Mycobacterium CM and AS (Hain Life Science, Germany) based on manufacturers protocol. Isolates which could not be identified beyond genus level by line probe assays were subjected to partial sequencing of four highly conserved genes by BigDye Terminator cycle sequencing chemistry (Applied Biosystems, USA). The hyper variable region of *16S-rRNA* (645–655bp), *rpoB* (342bp), *hsp65* (439bp), 16S-23S ITS region (480bp) genes were sequenced based on previously validated primers [12–15].

Data analysis

Primary sequence base calling was carried out by using Sequence Analysis software v5.3.1 (Applied Biosystems, USA) followed by sequence assembly in Seq Man Pro V-15 (DNA STAR, USA). Assembled sequences were later subjected to BLAST analysis in NCBI GenBank and EzTaxon (http://www.ezbiocloud.net/identify) online data base. A stringent similarity index of ≥99-100% was kept with the Type strain in GenBank and EzTaxon. Isolates remained without a proper identity after performing sequencing of all the four tested genes were assigned as *Mycobacterium* species. Basic statistical data analysis was carried out by using SPSS V20.0 software package (IBM, USA).

Results

Study population

Overall, 52 culture confirmed NTM cases were identified during the study period of 12 months. Children aged between 11–14 years were predominant (44.3%) with a major proportion of Saudi nationals (86.5%). Non-Saudi population included only 7(13.5%) cases (3 extrapulmonary and 4 pulmonary). A higher proportion of boys (59.6%) were evident compared to girls. The Central (48.1%) and Western (36.6%) provinces of the country reported with majority of incidence cases. Smear positivity was observed in 61.5% of cases. Extra-pulmonary sites of infection were commonly observed than pulmonary infections (73.1% versus 26.9%). 34/44 relevant cases received treatment mostly fine needle aspiration, surgical incision or antibiotic therapy (Table 1). Among the extrapulmonary cases, lymph nodes (55.3%) were mostly affected followed mainly by skin and soft tissues (21.1%) and blood (13.2%) (Table 1).

Species spectrum of NTM isolates

The species spectrum showed 11 different species including three rare species. The slow growing species *M. simiae* (30.8%) was predominant and followed by the fast growing species *M. abscessus* (23.1%) and *M. fortuitum* (11.5%). Six cases were observed with rare species such as *M. monacense* (three cases), *M. riyadhense* (two cases) and *M. kubicae* (one case) (Fig. 1).

The figure shows 11 NTM species identified by line probe assay and multiple housekeeping gene sequencing.

Pulmonary NTM diseases

The pulmonary groups included 14 cases with a mean age of 9.8 years. Pulmonary NTM species diversity showed nine different species mainly *M. abscessus*, *M. simiae*, *M. intracellulare*, *M. gordonae* and *M. monacense*. Use of ATS/IDSAguidelines for NTM pulmonary disease revealed 42.8% cases as clinically relevant. However, all the five cases with isolation of NTM from sputum did not meet the ATS/IDSA criteria and therefore classified them into pulmonary colonization. Pulmonary cases showed different comorbid conditions such as genetic disorders like congenital neutropenia, severe combined immunodeficiency (SCID), chronic

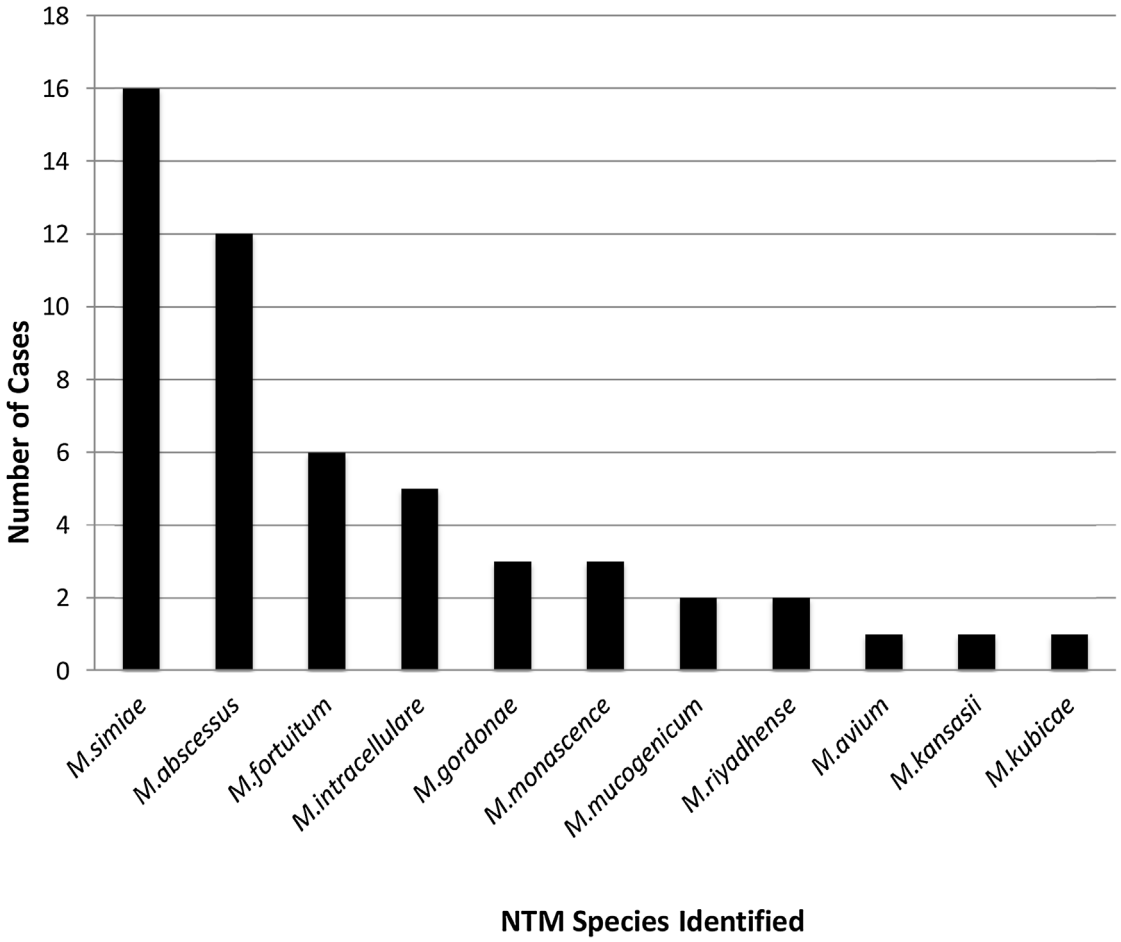

Fig. 1. Species diversity of NTM isolates from Saudi Arabian children.

granulomatous disease (CGD), Bare lymphocyte syndrome (BLS), Eisenmenger pulmonary hypertension (EPH) and CD3 deficiency. Bronchiectasis (28.6%) was another common risk factor among pulmonary cases (Tables 2 and 3).

Extrapulmonary NTM diseases

Lymphadenitis (40.4%) was the most common clinical manifestation caused by NTM's in the current study cohort with a mean of age as 6.4 years. Cervical lymphadenitis (57.2%) was predominant followed by submandibular (14.3%), inguinal (9.5%), preauricular (9.5%) and axillary (9.5%) lymph nodes. *M. simiae* (47.6%) and *M. abscessus* (23.8%) were the major causative agents of lymphadenitis. *M. riyadhense*, an emerging rare species was responsible for lymphadenopathy in an eight year old boy who suffered from cystic fibrosis. Another rare species, *M. kubicae* caused cervical lymphadenitis in a 13 year old Saudi boy with vitamin D deficiency and juvenile diabetes. Overall, 5(23.8%) cases were observed with genetic disorders and 7(33.3%) cases were immunocompetent in this group (Table 3). Among 17 'other' cases, 8(47%) were reported with an infection in skin or soft tissues. In addition, 5(29.4%) cases with mycobacteremia, 3(17.6%) with gastrointestinal and 1(5.9%) with genitourinary infections were also observed. *M. simiae*, *M. abscessus* and *M. fortuitum* were mostly isolated from this group. A rare event of ascites caused by *M. monacense* in an 11 year old girl suffering from Alport syndrome was observed. Genetic disorders were less frequent in this group; on the other hand five cases were immunocompetent (Tables 2 and 3).

Discussion

To the best of our knowledge, this is the first study in Saudi Arabia which analyzed pediatric NTM infections using a systematic approach and a nationwide coverage. Although total cases were limited to 52, the clinical relevance of 44(84.6%) cases which caused pulmonary or extra-pulmonary diseases showed considerable threat in the country caused by this pathogenic bacterial group. Demographical data showed a predominance of Saudi nationals and male gender. An increased prevalence of Saudi nationals compared to immigrant populations among NTM infected cases in the country was previously reported, although reason for this elevation is still unclear [4–6]. The Saudi Arabian population aged below 14 years is 78.2% compared to their non-Saudi counterparts, and that may probably reflect in total NTM incidences report. It is worth mentioning that immunosuppressive conditions such as carcinomas, autoimmune diseases, genetic diseases and various congenital disorders are relatively high among the Saudi population [16–18]. This may explain the possible vulnerability of Saudi population to NTM diseases. On the other hand, 23.1% of total cases in the study were defined as immunocompetent. Among 76.9% cases with single or multiple comorbid conditions, 47.5% cases showed either genetic or congenital disorders or both. To date, no studies systematically analyzed the association of confounding factors which may cause vulnerability to NTM diseases in Saudi Arabian pediatric population.

Saudi Arabia administers mandatory BCG vaccination by birth with a current coverage of 98%. However, data are unavailable on the efficacy and promised protection of BCG vaccination against

Table 1
Demographical and clinical summary of 52 pediatric NTM cases.

Parameters	No (%)
Age Groups (years)	
0–5	14(27)
6–10	15(29)
11–14	23(44)
Nationality	
Saudi	45(87)
Non-Saudi	7(13)
Gender	
Male	31(60)
Female	21(40)
Geographical origin	
Southern	2(4)
Central	25(48)
Western	19(36)
Eastern	6(12)
Clinical Characteristics	
AFB/Auramine-Rhodamine Smear	
Positive	32(61)
Negative	20(39)
BCG vaccinated	51 (98)
Treatment outcome	
[a]Cured/Treated	34(66)
Relapsed	1(2)
Not Treatment received	8(15)
Not Available	9(17)
Site of Infection	
Pulmonary	14 (27)
Extra-pulmonary	38(73)
Lymphnode	21(55.3)
Skin and soft tissues	8(21.1)
Blood	5(13.2)
Peritoneum	2(5.2)
Ascitis	1(2.6)
Genitourinary	1(2.6)

[a] Treated by drug therapy or fine needle aspiration or surgical excision.

Table 2
Summary of 12 immunocompetent pediatric NTM cases.

Cases	Age (years)	Gender	Nationality	Site of infection	Species
1	11	M	Saudi	Cervical LN[a]	*M. simiae*
2	6	F	Pakistani	Cervical LN	*M. simiae*
3	12	M	Saudi	Cervical LN	*M. simiae*
4	2	M	Saudi	Cervical LN	*M. simiae*
5	11	M	Eritrean	Axillary LN	*M. abscessus*
6	6	F	Saudi	Cervical LN	*M. simiae*
7	7	M	Saudi	Preauricular LN	*M.intracellulare*
8	9	M	Saudi	Skin	*M. simiae*
9	14	M	Saudi	Skin	*M. abscessus*
10	12	M	Saudi	Skin	*M. fortuitum*
11	8	F	Egyptian	Pulmonary[b]	*M. fortuitum*
12	13	M	Pakistan	Pulmonary[b]	*M. gordonae*

[a] Lymphnode.
[b] Clinically not relevant (colonizer) based on ATS/IDSA guidelines.

NTM infections in the Saudi pediatric population. A recent meta-analysis assessed the protective effectiveness of BCG vaccination against lymphadenitis and skin infections among pediatric population. The promising findings showed, BCG vaccination protected children against lymphadenitis and skin infection (particularly Buruli ulcer) [19]. In addition, other previous international studies also established a protection by BCG vaccination in many localized diseases caused by NTM's [20,21]. Therefore, we assume that BCG vaccination might give protection to children up to certain extent also in Saudi population. This finding corroborates with the evidence that only limited immunocompetent cases (22.7%) in our study group were observed with clinically relevant infections.

The predominance of boys in the Saudi Arabian cohort is an opposite trend compared to other part of the world, where girls infected more by various NTM species [22,23]. However, previous studies of mycobacterial infections in local population showed similar trend of male gender predominance [4–6]. Reasons behind this increased gender predominance are still unclear. Interestingly, congenital disorders particularly primary immunodeficiency diseases have been found comparatively high among boys (53% versus 47%) with a median of age 17 months. Similarly, findings of a previous study on 502 cases with primary immunodeficiency reflected the current findings as it showed predominance of male (63.1%) particularly with immunodeficiency associated with X linked genes and this could probably explains the different sex ratio of male [16]. Therefore, regardless of the BCG vaccination, male children were more affected than females in the current study. Predominance of male gender with comorbid conditions particularly among adult population was evidenced in other world regions [24].

Central and Western provinces reported with the highest incidence (84.7%). This may be due to the higher population density of these two provinces with the presence of abundant water and farmland sources. In addition, possibilities of hospital based transmission through common water sources or contaminated medical equipments also could not be ruled out. The species diversity showed predominance of slow growing *M. simiae* and rapid growing *M. abscessus* together in 53.8% of study isolates. Presence of *M. abscessus* even among immunocompetent patients in the country as an etiological agent of pulmonary and extra-pulmonary diseases was previously reported [4,6,25]. However, presence of *M. simiae* as the most common species is a new finding, because to date such a large level isolation was not reported. Furthermore, the isolation of *M. simiae* was not restricted to single laboratory or city, therefore chances of institutional outbreaks or laboratory contamination was limited. Three rare NTM species (*M. monacense*, *M. riyadhense*, *M. kubicae*) also were reported causing both pulmonary and extra-pulmonary diseases in concordance with recently reported emergence of rare NTM species in Saudi Arabia [5].

The most common clinical manifestation among the Saudi Arabian pediatric cohort was lymphadenopathy, which is consistent with previous studies from other global region [22,26,27]. Cervical lymph nodes (57.1%) were the most commonly affected although submandibular, preauricular, inguinal and axillary lymph nodes were involved. On the other hand, several previous studies also showed similar finding that cervico-facial lymph nodes are mostly infected by different NTM species in children [23,27]. *M. simiae* was the most common etiological agent of lymphadenitis in Saudi Arabian children. This finding is incongruous with previous global studies which showed *M. avium-intracellulare complex*, *M. scrofulaceum*, *M. haemophelium* and, *M. lentiflavum* were mostly causing lymphadenitis in children [26,28]. However, few recent studies reported the emergence of *M. simiae* as an establishing pathogen in the Middle East and other countries [29,30]. Among 17 cases of other extra-pulmonary sites, skin and soft tissue infections were the most common particularly among children above eight years. *M. abscessus* caused the majority of skin infections. Previous studies reported the role *M. abscessus* as an emerging etiological agent for skin and soft tissue infections [31]. This study showed five cases of mycobacteremia mainly caused by *M. simiae* and *M. mucogenicum*. Generally mycobacteremia is an uncommon event among children and reported more among pediatric HIV positive cases caused by *M. tuberculosis*. The current study cohort was free from any HIV positive children. Nevertheless, all the cases with mycobacteremia had either leukemia or other blood disorders as a comorbid condition. In addition, 4 of 5 cases were reported with central venous catheters and resembled previous findings which associated catheterization with mycobacteremia [32].

Involvement of gastrointestinal system was reported in three cases. A rare event of ascites caused by *M. monacense* was reported in an 11 year old girl suffering from Alport syndrome. A similar case of ascites in a 52 year old male patient also had been recently

Table 3
Summary of 40 pediatric NTM cases with known co-morbid conditions.

Cases	Age (years)	Gender	Nationality	Site of Infection	Species	Comorbid conditions
1	0.9	M	Saudi	Cervical LN[a]	*M. abscessus*	SCID[b]
2	1	F	Saudi	Cervical LN	*M. fortuitum*	Congenital neutropenia
3	4	F	Saudi	Submandibular LN	*M.intracellulare*	CGD[c]
4	8	F	Saudi	Cervical LN	*M. simiae*	IgA Nephropathy
5	4	M	Saudi	Cervical LN	*M.intracellulare*	Bronchiectasis
6	13	M	Saudi	Cervical LN	*M. abscessus*	Cystic fibrosis
7	1	M	Saudi	Submandibular LN	*M. simiae*	CGD
8	2	M	Saudi	Preauricular LN	*M. simiae*	Congenital neutropenia
9	3	F	Saudi	Inguinal LN	*M. abscessus*	Congenital heart disease
10	13	M	Saudi	Cervical LN	*M. kubicae*	Diabetes mellitus, VDD[d]
11	2	M	Saudi	Inguinal LN	*M. abscessus*	Acute myeloid leukemia
12	8	M	Saudi	Cervical LN	*M. riyadhense*	Cystic fibrosis
13	14	M	Pakistani	Axillary LN	*M. simiae*	SLE[e]
14	5	M	Saudi	Submandibular LN	*M. simiae*	Bronchiectasis
15	11	F	Saudi	GIS[f]	*M. monacense*	Alport syndrome
16	12	M	Saudi	Skin	*M. fortuitum*	Asthma, VDD[g]
17	8	F	Saudi	Blood	*M.mucogenicum*	Pneumonia, CML[g]
18	3	M	Saudi	Blood	*M. simiae*	Acute myeloid leukemia
19	3	F	Saudi	Blood	*M. simiae*	Acute myeloid leukemia
20	8	M	Saudi	Blood	*M. mucogenicum*	CML
21	11	M	Saudi	Blood	*M. simiae*	Congenital neutropenia
22	12	M	Saudi	Skin	*M. abscessus*	Cystic fibrosis
23	11	M	Saudi	Skin	*M. abscessus*	Bronchiectasis
24	4	M	Saudi	Skin	*M. gordonae*	SLE
25	13	M	Saudi	Skin	*M. abscessus*	MSMD[h]
26	12	M	Saudi	GIS	*M. abscessus*	COPD[i], peritoneal dialysis
27	11	F	Saudi	GIS	*M. fortuitum*	End stage renal disease
28	12	M	Saudi	Gastric aspirate	*M. simiae*	Congenital neutropenia
29	6	F	Saudi	GUT[j]	*M. simiae*	Acute myeloid leukemia
30	8	F	Saudi	Gastric aspirate	*M. monacense*	CD3 Deficiency
31	8	M	Saudi	Gastric aspirate	*M. riyadhense*	Congenital neutropenia
32	3	M	Saudi	Gastric aspirate	*M. intracellulare*	SCID
33	6	F	Saudi	BAL	*M. abscessus*	Bronchiectasis, EPH[k]
34	7	F	Saudi	Gastric aspirate	*M. monacense*	CGD,VDD
35	11	F	Chad	Sputum[l]	*M. gordonae*	Bronchiectasis
36	14	M	Saudi	Tracheal aspirate[l]	*M. simiae*	BLS[m], CF[n]
37	14	F	Saudi	Sputum[l]	*M. kansasii*	CML
38	12	M	Egyptian	Sputum[l]	*M. intracellulare*	Bronchiectasis, VDD
39	13	M	Saudi	Sputum[l]	*M. avium*	Pneumonia
40	9	M	Saudi	Tracheal Aspirate[l]	*M. abscessus*	Bronchiectasis

[a] Lymphnode.
[b] Severe combined immune deficiency.
[c] Chronic granulomatous disease.
[d] Vitamin D Deficiency.
[e] Systemic lupus erythematosus.
[f] Gastrointestinal system (peritoneal or ascitic fluids).
[g] Chronic Myeloid Leukemia.
[h] Mendelian susceptibility to mycobacterial disease.
[i] Chronic obstructive pulmonary disorder.
[j] Genitourinary tract.
[k] Eisenmenger pulmonary hypertension.
[l] Clinically not relevant (colonizer) based on ATS/IDSA guidelines.
[m] Bare lymphocyte syndrome.
[n] Cystic fibrosis.

reported in Saudi Arabia [5]. The other two gastrointestinal isolates were *M. abscessus* and *M. fortuitum*. The *M. abscessus* was isolated from the peritoneal fluid of a 12 year old boy undergoing peritoneal dialysis.

M. fortuitum was also isolated from peritoneal fluid of an 11 year old girl who suffers from end stage renal disease. Similar peritonitis caused by both *M. abscessus* and *M. fortuitum* were sporadically identified among patients who undergo continuous ambulatory peritoneal dialysis (CAPD) [33].

Pulmonary NTM cases were 26.9% in the study compared to extra-pulmonary sites of infections. Clinical relevance (42.8%) based on ATS/IDSA guidelines was relatively high compared to previous studies (25–33.5%) [34,35]. However, a scarcity of data on analyzing the clinical relevance of pediatric NTM pulmonary diseases still exists globally. Eight different species were observed among the pulmonary cases such as *M. abscessus*, *M. kansasii*, *M. fortuitum* and *M. avium* complex. In addition, two cases of *M. monacense* and one case of *M. riyadhense* were also noticed with conformed clinical relevance. To the best of our knowledge, these are the first cases of *M. monacense* and *M. riyadhense* causing clinically relevant pulmonary disease in children.

The study had some limitations particularly related to design. The study was an exploratory model, therefore deep population based or epidemiological analysis was restricted. As the first nationwide exercise, objectives were made to find only existing definite cases for 12 months rather than screening all presumptive TB/NTM or culture negative cases. Clinical follow up of pulmonary cases reported with one time isolation or colonization was not under the scope of the study. Therefore, comprehensive social and clinical data analysis including treatment management was limited.

Conclusions

To conclude, diverse pulmonary and extrapulmonary diseases are caused by common and rare NTM species among Saudi Arabian pediatric population aged from 0 to 14 years. However, majority of these pediatric cases hosts different genetic or congenital disorders or other co-morbid conditions. The large NTM species diversity among pediatric population reflects the similar presence of various pathogenic species among adult Saudi Arabian population. The awareness on threats exerted by new and rare NTM's must be periodically given to clinicians, otherwise the current diagnostic conundrum will be unlikely to change. The substantial volume of current research gaps on this subject must be addressed immediately in the country. An immediate requirement to develop right NTM disease management guidelines for the country is highly warranted.

Funding

This work was supported by King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia (Grant code # 12-MED-3172-20).

Competing interests

None declared.

Ethical approval

The study has been reviewed and approved by the Office of Reseach Affairs in King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

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