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Non Tuberculous Cutaneous Mycobacteriosis in a primary school in Rome: epidemiological and microbiological investigation

F.P. D'Ancona[1], E.E. Kanitz[1,2], L. Marinelli[3,7], J.L. Sinagra[4], G. Prignano[4], C. Cerocchi[5], L. Bonadonna[1], E. Tortoli[6], B. Capitanio[4], A. Cottarelli[3], M. De Giusti[3,7]

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Abstract

During the school years 2009-2010 and 2010-2011 a total of 25 cases of Non Tuberculous Cutaneous Mycobacteriosis (NTCM) were notified in children attending the same school with a swimming pool in Rome. Environmental microbiological and epidemiological investigations (only for suspected outbreaks in 2009-2010) were conducted.

We screened students with skin lesions, and environmental samples were collected from the school area and the swimming pool.

During the school year 2009-10 18 cases were clinically identified among 514 primary school children (3.50%) and all cases attended the swimming pool. Only 2 out of 18 cultures were positive for Mycobacterium chelonae complex (Group III, M. abscessus). Attack Rate for swimming pool use was 13,10% (17/130), with a Relative Risk 54,70 (95% CI: 9,4 - ∞).

In February 2011 additional 7 cases of cutaneous NTM among children - who attended the same primary school and swimming pool were notified to the local public health authority followed by environmental microbiological investigation.

Environmental samples were positive for NTM but not for M. abscessus.

Mycobacteria are not included in water-quality criteria in Italy for this reason it is important to collect evidences of NTM cases caused by these infrequent pathogens, to be able to perform rapid risk assessment and to identify the best practices in prevention and management of such a risk.

Introduction

Non Tuberculous Mycobacteria (NTM) are becoming increasingly important cutaneous pathogens as the number of susceptible patients increases (1). These infections are caused by different mycobacterial species originating from the

[1] *National Institute of Health, Rome, Italy*
[2] *European Programme of Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control, Stockholm, Sweden*
[3] *Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy*
[4] *San Gallicano Dermatologic Institute IRCCS, Rome, Italy*
[5] *Local Public Health authorities*
[6] *Regional Reference Centre for Mycobacteria, Careggi University Hospital, Florence, Italy*
[7] *Accademia Romana di Sanità Pubblica*

environment (water, soil, plants, and animals). Various factors influence the clinical manifestations of the cutaneous infection: the immunological situation of the patient, the degree of cutaneous impairment and the extent of the contact with the contaminated environment.

M. abscessus, also known as a subspecies of *M. chelone*, is a rapid-growing Mycobacterium (Runyon IV) that is ubiquitous to water, soil and dust (2, 3) and has an optimum growth at 28-30° (4). Infection occurs through contaminated water, infected fish, traumas or surgical wounds; there is no evidence of person-to-person transmission (3). It is associated with localized cutaneous disease and mainly affects hands, elbows, knees and feet (2, 5). Painful granulomatous lesions appear a few weeks after infection, and later progress into ulcerated nodules. There is no standardized treatment, and the lesions can take many months to heal and can also be self-limiting in children. Serious complications can occur in immunocompromised individuals including pulmonary or disseminated disease, but they are rare in basically healthy individuals.

M. abscessus is often involved in post-traumatic or post-surgical infections and has caused outbreaks associated with cosmetic procedures. The largest outbreak due to *M. abscessus* was associated with a wading pool and occurred in Canada in 2003, involving 85 cases (5).

In February 2010, a dermatology hospital in Rome area notified the local Public Health Authorities about an unexpected occurrence of 18 clinical cases of NTCM among children attending the same primary school with a swimming pool in Rome. An investigation was launched in February 2010, which was conducted by a large team consisting of the local Public Health Authorities, epidemiologists, dermatologists, microbiologists and water pollution experts. The objectives of the investigation were to confirm the diagnosis and the suspected outbreak, to identify cases and to discover the source of infection and to implement control measures.

The swimming pool was treated in April 2010 according to the WHO-guidelines for recreational waters (6). No cases were reported in the following 6 months until February 2011, when other seven cases of NTCM among children who attended the same swimming pool were notified at the local public health authority.

Methods

This is an observational study following the notification of cases of NTCM among children attending primary school in Rome.

The case definition was formulated as follows: a case was a person attending the school in 2009-2010, with skin lesions based on clinical examination (*suspect case*), plus histopathology results of skin lesions (*probable case*), and/or positive culture (*confirmed case*).

For microbiologic investigations, the purulent material from swabs or biopsies was inoculated, after appropriate digestion and decontamination with antibiotic mixture (BBL™ MGIT™ PANTA™ Becton, Dickinson and Company, USA) and BBL™ MGIT™ OADC enrichment onto the Mycobacterial Growth Indicator Tube (MGIT, BD) and seeded on culture media (Lowenstein-Jensen medium, BD Becton, Dickinson and Company, Sparks, Maryland, USA). Media were incubated at 36 ±1°. Colonies were stained with Ziehl Nelsen stain for acid-fast bacteria and processed for strain identified by the reverse hybridization method (Inno-Lipa Mycobacteria v2 Innogenetics, Belgium) and kit GenoType CM/AS (Arnika, HainLifescience GmbH, Nehren).

Active case finding was carried out during March and April 2010. A team - including the dermatologist - went to the school on four occasions, which were previously

announced to the parents through the director of the school. 428/514 (83%) primary school children participated in the screening for lesions on their hands, arms, feet, legs and back; the remaining children's parents had not given their consent for the children to be looked at.

A questionnaire was handed to parents of first identified cases to investigate symptoms and exposures. Information on participation to a weekly swimming course in the pool of the school was available for all school children.

Environmental samples were obtained February-April 2010 and April 2011, during the second wave of notification of NTCM, from school and swimming pool water, tap water, pool edge, shower tray and shower mat. The environmental samples were obtained according to the literature method and media were incubated at 36 ±1° up to 6-10 weeks (7).

Statistical analysis

Univariate analysis and Exact Poisson Regression were performed including all children who had participated in the screening (428/514 children) to estimate the increased NTCM risk among children with documented use of swimming pool. The statistical analyses were performed using SPSS (Release 12.0).

Results

The suspected *M. abscessus* outbreak in a primary school in Rome involved 18 cases (12 girls, 6 boys), with dates of onset between November 2009 and February 2010 and 17 out of the 18 cases had taken weekly swimming lessons in the pool of the school.

The diagnosis is based on clinical examination, and in seven cases on histopathological examination of skin biopsies. All cases were diagnosed through the main dermatology hospital in Rome (which is nearby to the school); no cases were reported outside the school. Microbiological confirmation of *M. abscessus* were obtained from 2 out of 18 children, culture from the other patients did not confirmed any results.

NTCM cases were aged from three to ten years, with a median age of seven years (data not shown). None of the cases suffered from any underlying bad health conditions. All patients presented with lesions on their hands, arms, feet and back. All cases were treated with clarithromycin for a minimum period of six weeks, and recovered without complications or requiring surgical interventions. There was no evidence for person-to-person transmission; cases were distributed across all classes in the school, and there were no clusters within families.

The questionnaire obtained from the parents of 9 out of 18 patients (50% of responders) did not reveal common exposures except participation in a weekly swimming course in the school since the beginning of the school year in September 2009.

Participation in such a swimming course was recorded for all school children and univariate analysis was performed including all children involved in the screening (428/514 children). Of 428 children, swimming pool use was not known for two children (one case and one non-case, and they were also excluded from the analysis). Of the remaining 426 children, 130 children had taken a weekly swimming course (30.50%), including all 17 diagnosed positive (Attack rate AR_{pool}= 13.00%). The Risk Ratio estimate (obtained by Exact Poisson regression) was 54.70 (9.4-∞).

Qualitative analysis of the environmental samples during the primary suspected outbreak revealed the presence of *M. mucogenicum*, *M. phocaicum*, *M. llatzerense, M. chelonae*, *M. fredericksbergense* and *M. gordonia terra* in the water of the swimming pool water and in the municipal tap water but the presence of *M. abscessus* in

Table 1 - Mycobacteria detection from environmental samples during the two suspected outbreaks in the school years 2009-2010 and 2010-2011

Sampling site	Results 2009-2010	Results 2010-2011
Swimming pool	*M. mucogenicum*[(a)]	*Mycobacterium* genus[(c)]
	M. phocaicum[(a)]	*Mycobacterium* genus[(d)]
Shower of the swimming pool	negative	*M. fortuitum*[(e)]
		Mycobacterium genus[(f)]
School	*M. llatzerense*[(b)]	Negative
	M. chelonae[(b)]	
	M. llatzerense[(b)]	
	M. gordonia terrae[(b)]	
	M. fredericksbergense[(b)]	
	M. fredericksbergense[(b)]	

[(a)] not specified; [(b)]Municipal tap water of Rome; [(c)]air-water interface swimming pool for water intake site; [(d)]tap water downstream of chlorination system; [(e)]women shower mat; [(f)]men shower mat.

the swimming pool could not be confirmed through this analysis (Table 1).

However, the risk factor analysis strongly pointed towards the swimming pool as a source of infection, in which *M. abscessus* was possibly present transiently, probably between October and December 2009. Therefore measures were implemented in April 2010 according to the WHO guidelines (6), including treatment of the swimming pool with thermal shocks, chlorination, cleaning of all surfaces and change of filters, since February 2011 when the local Public Health Authorities notified seven cases again and a second environmental sampling was carried out in the swimming pool.

Also in this occasion the correlation between the swimming pool and the outbreak caused by *M. abscessus* could not be confirmed because all the samples were negative for *M. abscessus* but the qualitative analysis revealed the presence of Mycobacterium genus in different samples, in particular, in air-water interface swimming pool for water intake site, tap water downstream of chlorination system and men shower mat and *M. fortuitum* in woman shower mat of the swimming pool (Table 1).

Discussion

We are reporting on two distinct suspected outbreaks linked to a swimming pool caused by the *M. abscessus* which occurred between October 2009- February 2010 and February 2011 in Rome.

All eighteen cases were children who attended a private primary school in Rome during the 2009-2010, and seventeen out of the eighteen cases had taken weekly swimming lessons in the pool of the school. We found a statistically highly significant association with the swimming pool,

although *M. abscessus* was not found in the pool water. None of the children had any underlying medical conditions, and all improved after a six-weeks treatment with clarithromycin.

Also in the second outbreak, seven new cases of NTCM occurred during the school year 2010-2011 and were suspected to be related to the same swimming pool. The environmental samples could not confirm the correlation between the use of the swimming pool and the onset of the infection because *M. abscessus* was not detected. However the presence of *M. fortuitum,* detected on the shower mat, can cause a similar skin disease (8), and it appears to be the most predominant etiology of cutaneous and soft tissue infections in young individuals (9) and its presence may represent a growing problem for the swimming pool.

However there were several limitations of this observational study: due to the delay of the start of the investigation, it was not possible to collect detailed information on exposure. Therefore no comprehensive analytical study could be carried out. For the same reasons, the delayed sampling of the swimming pool water and other sites at the school yielded negative results, *M. abscessus* could not be found in the environmental samples.

However, the epidemiological investigation and the risk factor analysis strongly pointed towards the swimming pool as a source of infection, in which *M. abscessus* could be present transiently.

Mycobacteria are not included in water-quality criteria in Italy (10), and although it is known that standard chlorination of drinking and leisure waters are not sufficient to eliminate Mycobacteria, the Public Health Authorities were unable to enforce temporary closure of the swimming pool till evidence based on analytical results.

Being still few the studies about the prevalence of Mycobacteria in swimming pools, and it would be advisable to increase investigations to estimate the magnitude of the problem. Moreover we know that current strategies of disinfection are inefficient against Mycobacteria (11) and more knowledge of NTM ecology could contribute to identify best practices for rapid risk assessment and management of the pool and recreational water that represent important sources of human NTM infection.

Riassunto

Micobatteriosi cutanea non tubercolare in una scuola romana: indagine epidemiologica e microbiologica

Durante gli anni scolastici 2009-2010 e 2010-2011 sono stati notificati 25 casi di micobatteriosi cutanea non tubercolare (NTM) in bambini frequentanti stessa scuola e stesso impianto natatorio situati in Roma. È stata condotta una indagine microbiologica ambientale ed un'attenta indagine epidemiologica limitatamente alla sospetta outbreaks 2009-2010.

Sono stati considerati studenti con lesioni cutanee, e sono stati prelevati campioni ambientali sia dalla scuola sia dalla piscina.

Durante l'anno scolastico 2009-2010 diciotto casi su 514 bambini della scuola (3,50%) sono stati identificati clinicamente e tutti i casi frequentavano la piscina. Solo 2 colture su 18 sono risultate positive per *Mycobacterium chelonae* complex (Group III, *M. abscessus*). L'Attack Rate per l'uso della piscina è stato il 13,10% (17/130), con un RR di 54,7 (95% CI: 9,4 - ∞).

Nel febbraio 2011 altri sette casi di micobatteriosi cutanea NTM, tra i bambini frequentanti la stessa scuola e piscina, sono stati notificati all'Autorità Sanitaria Locale a cui ha fatto seguito una indagine microbiologica ambientale.

I risultati dei monitoraggi ambientali hanno confermato presenza di NTM ma non di *M. abscessus.*

In Italia i micobatteri non sono inclusi nei criteri di qualità dell'acqua, per questa ragione è importante acquisire evidenze di casi di micobatteriosi NTM causate da questi patogeni poco frequenti per essere in grado di impostare un rapido risk assessment e identificare le migliori pratiche di prevenzione e gestione di tale rischio.

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Corresponding author: Prof. Maria De Giusti, Department of Public Health and Infectious Diseases, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
e-mail: maria.degiusti@uniroma1.it