Official journal of the Spanish Society of Chemotherapy

Original

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Historical evolution of the diseases caused by non-pigmented rapidly growing mycobacteria in a University Hospital

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Article history
Received: 22 March 2019; Revision Requested: 14 May 2019; Revision Received: 20 May 2019; Accepted: 22 May 2019

ABSTRACT

Introduction. Non-pigmented rapidly growing mycobacteria (NPRGM) are a group of organisms of increasing interest due to the growing number of potential patients and the difficulties for a proper treatment in many of them. However, the evolution of these diseases in a long period of time and its evolutionary changes has been described only in a scanty number of reports.

Material and methods. We performed a retrospective study between January 1st 2004 and December 31st 2017 in order to evaluate the clinical significance and types of diseases caused by NPRGM. Patients with isolates of NPRGM during this period were selected for the study, and clinical charts were reviewed using a predefined protocol.

Results. During this period we identified 59 patients (76 clinical samples) with isolates of NPRGM, with 12 cases of clinical disease and one patient with doubtful significance (including 6 respiratory tract infections, 2 catheter infections, 1 skin and soft tissue infection, 1 disseminated infection, 1 conjunctivitis, 1 prosthetic joint infection and 1 mastitis). Fifty percent of *M. chelonae* isolates, 37.5% of *M. abscessus* isolates and 23.33% of *M. fortuitum* isolates were clinically significant. None of the isolates of other species were significant.

Conclusions. Most isolates in respiratory samples were contaminants/colonizations. *M. abscessus* was the main etiological agent in respiratory syndromes, whereas *M. chelonae* and *M. fortuitum* were more frequently associated with other infections, especially clinical devices and skin and soft tissue infections.

Keywords: Non-pigmented rapidly growing mycobacteria; *Mycobacterium abscessus*; *Mycobacterium chelonae*; *Mycobacterium fortuitum*; clinical significance; historical evolution; epidemiology.

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Evolución histórica de las enfermedades causadas por micobacterias no pigmentadas de crecimiento rápido en un Hospital Universitario

RESUMEN

Introducción. Las micobacterias no pigmentadas de crecimiento rápido (MNPCR) son un grupo de organismos de interés creciente debido al número cada vez mayor de pacientes potenciales y a las dificultades en el tratamiento. Sin embargo, el número de estudios que analizan la evolución de estos casos a lo largo de un periodo de tiempo largo es escaso.

Material and métodos. Se realizó un estudio retrospectivo entre el 1 de enero de 2004 y el 31 de diciembre de 2017 para evaluar el significado clínico y los tipos de enfermedades causados por MNPCR. Se seleccionaron para ello aquellos pacientes con aislamientos de MNPCR, y se revisaron las historias clínicas mediante un protocolo predefinido.

Resultados. Se identificaron 59 pacientes (76 muestras) con aislamientos de MNPCR, de los cuales 12 presentaron enfermedad y uno tuvo un significado dudoso (incluyendo 6 infecciones respiratorias, 2 infecciones asociadas a catéter, 1 infección de piel y partes blandas, 1 infección diseminada, 1 conjuntivitis, 1 infección de prótesis osteoarticular y 1 mastitis). El 50 % de los aislamientos de *Mycobacterium chelonae*, el 37,5 % de *Mycobacterium abscessus* y el 23,33 % de *Mycobacterium fortuitum* fueron clínicamente significativos. Ninguno de los aislamientos de otras especies fue significativo.

Conclusiones. La mayoría de los aislamientos de muestras respiratorias resultaron ser contaminantes/colonizaciones. *M. abscessus* fue el principal agente etiológico en las infecciones respiratorias, mientras que *M. chelonae* y *M. fortuitum* fueron asociados con mayor frecuencia a otras

infecciones, especialmente infecciones de piel y partes blandas e infecciones asociadas a dispositivos biomédicos.

Palabras clave: micobacterias no pigmentadas de crecimiento rápido; *Mycobacterium abscessus*; *Mycobacterium chelonae*; *Mycobacterium fortuitum*; significado clínico; evolución histórica ; epidemiología.

INTRODUCTION

Non-tuberculous mycobacteria (NTM) are a group of opportunistic pathogens which are being increasingly recognized as a cause of infection [1]. They are also environmental organisms that can be found in many different ecosystems without public health implications [2].

NTM infections are an emerging phenomenon, mainly in the last decade [3]. It has been observed an increasing importance of infections caused by these organisms, both localized and disseminated, including also outbreaks and pseudo-outbreaks [4-5]. Among these, non-pigmented rapidly growing mycobacteria (NPRGM) are ubiquitous in nature and widely distributed in water, soil and animals [2, 6].

The three most important species of this group, regarding their clinical relevance, are *Mycobacterium fortuitum*, *Mycobacterium chelonae* and *Mycobacterium abscessus* [7]. However, there are many other species capable of causing human diseases such as *Mycobacterium mucogenicum*, *Mycobacterium immunogenum*, *Mycobacterium goodii*, *Mycobacterium peregrinum*, *Mycobacterium phocaium*, *Mycobacterium porcinum*, *Mycobacterium smegmatis* or *Mycobacterium wolinskyi* [7-9].

These microorganisms have the ability to form biofilms and this gives these organisms many advantages over the planktonic type of growth, as resistance to environmental aggressions and an increased resistance against disinfectants and antibiotics [10].

M. abscessus is one of the most frequently causative agents of nontuberculous mycobacterial pulmonary disease, often isolated in patients with underlying chronic lung diseases, like old tuberculosis scars, silicosis, bullae and other lung cavities where NTM can develop a biofilm. In recent times, patients with chronic bronchiectasis and cystic fibrosis have been found to be a target for NPRGM infections [11-13].

M. chelonae and *M. fortuitum* are frequently isolated in skin and soft tissue infections [14-15]. However, all these organisms can be isolated in other different clinical samples as a cause of many types of infection.

Here we report our experience with the diseases caused by these organisms isolated in our hospital during a 13-year period in order to compare these results with previous studies regarding these organisms.

MATERIAL AND METHODS

A retrospective study was performed to evaluate the clinical significance of the NPRGM. For this purpose, records dating from January 1st 2004 to December 31st 2017 from the mycobacteriology laboratory of the clinical microbiology department were reviewed. Patients with at least one isolate of NPRGM from clinical samples were selected for clinical charts review.

Sample processing and identification of bacterial isolates was performed following the internationally accepted protocols. The decontamination technique for all samples was

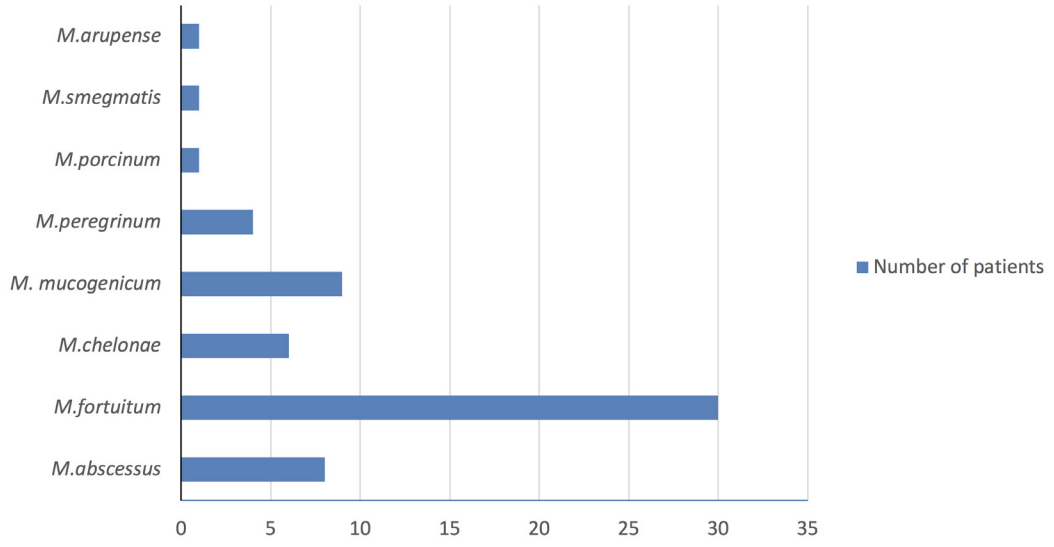

Figure 1 Number of patients with NPRGM

NPRGM: non-pigmented rapidly growing mycobacteria

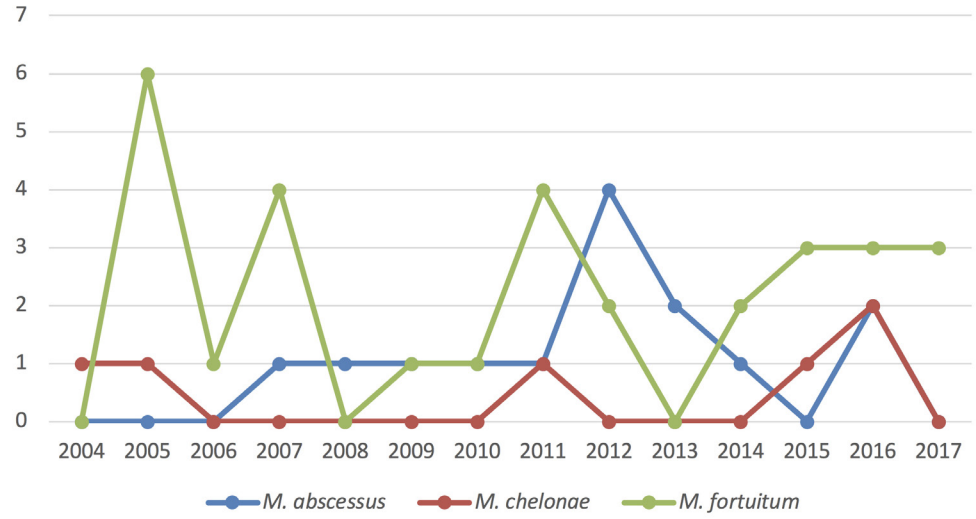

Figure 2 Evolution of cases of infection caused by NPRGM (patients/year)

NPRGM: non-pigmented rapidly growing mycobacteria

the N-acetyl-cysteine-NaOH protocol throughout all these years. After decontamination, all samples were inoculated onto Lowenstein-Jensen and Coletsos solid slants and were inoculated also in a liquid medium (MGIT 960 from 2004 to 2009, from BD, USA, VERSATREK (Biomérieux, France) from 2009 to 2016 and BacT/ALERT 3D (Biomérieux, France) from 2016 to date.

Mycobacterial isolates were identified using a commercial PCR identification test (GenoType CM/AS, Hain, Germany), and those isolates that could not be identified with this technique were sent to the Mycobacteria reference laboratory (Centro Nacional de Microbiología, Majadahonda, Spain). Antimicrobial susceptibility test was performed for all mycobacterial isolates with the broth microdilution reference technique [16].

On one hand, clinical charts were evaluated according to a predefined protocol that includes demographics, evaluation of risk factors (respiratory syndromes, HIV infection, immunosuppressive drug treatment and presence of biomaterials), clinical syndrome, treatment and outcome. Criteria from the ATS [17] for interpretation of an isolate were followed to determine the clinical significance of each case. On the other hand, we considered a case of doubtful clinical significance when it presented with clinical signs of infection in the absence of other possible causes, and showed improvement after treatment with antimicrobial therapy, but did not fulfill the microbiological criteria. The Clinical Research Ethics Committee of our hospital approved the study (registration number EO137-18_FJD)

RESULTS

Growing of NPRGM was observed in 76 clinical samples of 59 patients from January 1st 2004 to December 31st 2017.

Isolated mycobacteria were *Mycobacterium fortuitum* (30 patients), *Mycobacterium abscessus* (8 patients), *Mycobacterium mucogenicum* (9 patients), *Mycobacterium chelonae* (6 patients), *Mycobacterium peregrinum* (4 patients), *Mycobacterium porcinum* (1 patient), *Mycobacterium smegmatis* (1 patient) and *Mycobacterium arupense* (1 patient) (figure 1). One patient had two different NPRGM in two different samples. *M. fortuitum* was the most frequent isolated mycobacterium, with a sharply increase in 2005, 2007 and 2011. *M. abscessus* had a significant increase in 2012. *M. chelonae* was the less isolated mycobacterium with only 0 or 1 isolates per year, except in 2016 when it was isolated three times (figure 2).

Most of the samples were sputum and other respiratory samples (58 samples) followed by wound exudates and skin biopsies (5 samples), urine (3 samples), blood cultures (2 samples) and several other samples (8 samples).

Clinically significant cases appeared in 12 patients (20.3 %). One patient was classified as doubtful, and the rest of them were non-clinically significant cases. Syndromes and treatment of the patients with true or doubtful clinical significance are shown in table 1.

The clinical syndromes related to NPRGM include respiratory tract infections (6 cases), catheter infections (2 cases), skin and soft tissue infection (1 case), disseminated infection (1 case), conjunctivitis (1 case), prosthetic joint infection (1 case) and mastitis (1 case).

Regarding the clinical relevance of each species, 50% of the isolates of *M. chelonae*, 37.5% of *M. abscessus* and 23.33% of *M. fortuitum* were clinically significant. None of the isolates of other species were significant.

Table 1 **Characteristics of the cases of infection caused by NPRGM and the case with doubtful significance.**

Case	Year	Sex	Age	Underlying diseases	Syndrome	Positive samples	Acid-fast stain	Therapy	Species
1	2004	F	36	Chronic bronchopaty	Dysphonia	Laryngeal biopsy	Positive	IS+RI	*M. chelonae*
2	2005	F	45	NO	Mastitis	Skin exudate	Negative	CI	*M. fortuitum*
3	2005	M	55	Multiple myeloma	Catheter infection	Catheter exudate	Positive	AM+CI+CL	*M. chelonae*
4	2006	F	30	Depressive syndrome	Skin and soft tissue infection	Skin biopsy	Negative	CI	*M. fortuitum*
5	2007	F	51	HIV, Burkitt lymphoma	Disseminated infection	Blood cultures	Negative	CL+CO	*M. fortuitum*
6	2007	M	48	Multiple myeloma	Catheter infection	Catheter exudate	Negative	CO	*M. fortuitum*
7	2008-2013	M	45	HIV, Chronic respiratory insufficiency	Bronchiectasias	Sputum	Negative	CL	*M. abscessus*
8	2011	F	86	Lower eyelid myofibroblastic tumor	Conjunctivitis	Conjunctival exudate	Not performed	CL	*M. chelonae*
9	2012-2014	F	56	Chronic obstructive pulmonary disease	Bronchiectasias	Bronchial lavage	Negative	LE	*M. abscessus*
10	2012	F	80	NO	Arthritis	Bone prosthesis	Negative	CI+RI	*M. fortuitum*
11	2012	F	62	Alpha1-antitrypsin deficiency	Bronchiectasias	Sputum	Positive	NO	*M. abscessus*
12	2015	M	71	Chronic obstructive pulmonary disease	Bronchiectasias	Sputum	Negative	CI	*M. fortuitum*
13	2015	F	49	Asthma	Bronchiectasias	Bronchial lavage	Negative	CI+CO	*M. fortuitum*

NPRGM: non-pigmented rapidly growing mycobacteria; M: Male; F: Female; NO: No disease/Not received; AM: Amikacin; CI: Ciprofloxacin; CL: Clarithromycin; CO: Cotrimoxazole; IS: Isoniazid; LE: Levofloxacin; RI: Rifampicin.

When we focus on the underlying diseases in the clinically significant group chronic respiratory disease (6 cases), presence of malignancy (2 cases) and human immunodeficiency virus (HIV) disease (2 cases) were detected. There was 1 case of surgical infection related to shoulder prosthesis.

Acid-fast bacilli were detected in stains from samples in 4 of the significant and doubtful cases (33.33%). There was a clinically significant case (conjunctival exudate) in which the acid-fast stain was not performed. Interestingly, 2 samples of the non-significant group were also acid-fast stain positive.

Regarding the therapeutic actions in the clinically significant group, all the patients were treated with antimicrobial therapy except the doubtful case. There were 8 cases treated with monotherapy regimen (3 cases with ciprofloxacin, 2 cases with clarithromycin, 1 case with levofloxacin and 1 case with cotrimoxazol). Ciprofloxacin and clarithromycin were the mainly used antibiotics, either as monotherapy or in a combination antimicrobial regimen. In the implant-related infection it was necessary to remove the prosthesis in order to cure the infection. All the patients were cured, except 2 cases which are currently being under follow-up/control. One patient died due to other pathology. No resistances during therapy were detected.

DISCUSSION

NPRGM are usually considered environmental opportunistic pathogens. In our series we documented that only 20.34% of the isolates were clinically significant, compared to 30.8 % in a previous study [18]. This fact could be related to the increased number of respiratory tract isolates, probably due to the environmental nature of these bacteria. *M. abscessus*, *M. chelonae* and *M. fortuitum* have been usually associated with human diseases, while other members of the group are environmental isolates that cause human infections in rare cases [18-20]. *M. porcinum* has emerged in the last years as a species clearly related to human diseases, being involved in respiratory infections [21], but our only isolate has no role in the disease of this patient. Among other species, most clinically significant cases of *M. mucogenicum* isolates are involved in catheter-related infections [22]. *M. peregrinum* is a species included in the *Mycobacterium fortuitum complex*, but only a few cases of true infections have been reported, mainly related to surgical site infections and catheter-related infections [23]. *M. arupense* isolates have been related to pulmonary disease and osteoarticular infection [24-25]. In our series, all these species appeared to be colonizing organisms or contaminants, while the clinically relevant isolates belonged to the most common pathogens of this group: *M. fortuitum*, *M. abscessus* and *M. chelonae*.

According to the literature, the isolation of NPRGM has not a clear role in respiratory infection diseases such as chronic obstructive pulmonary disease (COPD) and bronchiectasis [19]. In these patients, the distinction between colonization and infection is a difficult clinical decision in most cases. *M. abscessus* is known to be a pathogen implicated in respiratory syndromes. It account for the majority of pulmonary infection cases in patients with underlying diseases like bronchiectasis,

cystic fibrosis or granulomatous diseases like sarcoidosis [12, 26-28]. Twenty eight percent of cystic fibrosis patients are affected by this species, being associated with increased morbidity and mortality, as well as with a rapid decline in lung function. Although it is not an absolute contraindication for lung transplantation, the pulmonary infection is associated with poor prognosis following this procedure [13, 27, 29].

In our series, most of NPRGM isolations from respiratory samples are not considered to be the major cause implicated in the pathology, but in patients with bronchiectasis, *M. abscessus* was the main isolated pathogen and all cases were treated with monotherapy, except one case that was considered of doubtful significance. This last case had a special clinical situation due to an alpha 1-antitrypsine deficiency, and the isolate was considered colonization because of the lack of symptoms, despite the fact that the organism was isolated from several different samples during a long time period.

The second species more frequently isolated in our series in respiratory samples was *M. fortuitum*. The respiratory infection caused by these mycobacteria is less common than *M. abscessus* disease, but there are cases reported in literature [30-31].

All of the biomaterial-related infections in our series required a combined medical and surgical therapeutic approach. Surgical procedures consisted of implant removal (meshes, catheter, and other prosthesis). *M. fortuitum* was the most frequently isolated mycobacteria from these infections. The ability of rapidly growing mycobacteria to develop biofilms in different surfaces is well known [32-34]. This virulence factor makes almost impossible the eradication of this bacteria using only antimicrobial therapy because of the *in vivo* resistance of sessile organisms against the different antimicrobials [35], so biofilm removal is mandatory in these cases.

In skin and soft tissue infections *M. fortuitum* was the main etiologic agent in our series**.** Acupuncture, infected surgical equipment or tattoos have been established as risk factors to develop a NPRGM skin infection [36-37]. The water used in the sterilising processes seems to be the main source of contamination in many cases**.** Monotherapy regimen was the selected treatment in all cases for these infections.

Due to the fact that NPRGM are resistant to conventional antituberculous drugs, the treatment has to be directed through *in vitro* susceptibility testing [16], being clarithromycin and ciprofloxacin the most frequently selected antibiotics [38]. Despite the previously described development of resistance during monotherapy [39], we have not detected any case of such problem, probably due to the low bacterial load presented in most of the cases, which minimises the probability of selection of resistant mutants.

In conclusion, the major difficulty to evaluate the clinical significance of NPGRM resides in the fact that most of these isolates are regarded as a contamination. However, we observed in our series that the isolation of a specific NPGRM (*M. abscessus, M. fortuitum* and *M. chelonae)*, or in specific samples (respiratory samples, skin, soft tissue, and biomaterials) is almost always related to the clinical syndrome. In order to avoid the failure of the treatment, an adequate microbiological identification and susceptibility test is needed, which would allow to choose a correct antimicrobial therapy and management of patients.

FUNDING

None to declare

CONFLICT OF INTEREST

The author(s) declare(s) that they have no conflicts of interest

JE received conference payments from Heraeus and Sysmex, and travel grants from Pfizer and bioMérieux. No conflicts of interest for all other authors.

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