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Molecular identification using PCR-RFLP´s technique of Nontuberculous Mycobacteria (NTM), isolated from patients undergoing aesthetic procedures; series of 20 cases

Jorge Leonardo Mayorga-Garibaldi[1]*; Mariana Lizett Zárate Torres[2]; Jorge Arturo Mayorga-Rodríguez[3]; Jaime Palomares-Marín[4]; Yaxsier de Armas-Rodríguez[4,5]; Claudia Lizeth Cueto Aragón[6]

[1]Diagnostic and Research Unit in Medical Microbiology and Infectious Diseases, Mexico.

[2]Dermatologist, Private Practice, Mexico.

[3]Head of the Reference Center in Mycology (CEREMI), Dermatologic Institute of Jalisco, Mexico.

[4]University Center of Health Sciences, Department of Microbiology and Pathology, University of Guadalajara, Mexico.

[5]Department of Pathology, Hospital Center, Institute of Tropical Medicine, Mexico.

[6]Children´s Hospital, "Eva Sámano de López Mateos" Morelia, Michoacán, Mexico.

***Corresponding Author:**

Jorge Leonardo Mayorga-Garibaldi

Diagnostic and Research unit in Medical Microbiology and Infectious Diseases, Guadalajara, Jalisco, Sierra Nevada, Mexico.

Email: leonardo_maygar91@hotmail.com

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Abstract

Nontuberculous Mycobacteria (NTM) constitute a diverse group of bacteria in the genus Mycobacterium. These bacteria are distinguished from *M. tuberculosis* and *M. leprae* by their inability to cause tuberculosis or leprosy.

Materials and methods: A retrospective study was conducted from 2024 to 2025 with the objective of identifying patients who underwent cosmetic procedures and were infected with NTM.

Results: During the study period, a total of 20 patients undergoing cosmetic interventions were identified as having NTM infections. The diagnosis was performed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique.

Introduction

Nontuberculous Mycobacteria (NTM) constitute a diverse group of bacteria in the genus Mycobacterium. These bacteria are distinguished from *M. tuberculosis* and *M. leprae* by their inability to cause tuberculosis or leprosy. These bacteria are ubiquitous in the environment, found mainly in soil and aquatic environments [1,2]. Although NTM are generally of low pathogenicity, they have the capacity to cause opportunistic infections, especially in immunocompromised individuals [1,3].

NTM are classified into two main groups based on their growth speed: Slow-growing and fast-growing. Among slow-growing species, *Mycobacterium Avium* Cmplex (MAC) is the most prevalent and is a significant cause of Nontuberculous Mycobacterial Lung Disease (NTM-PD). Conversely, the *Mycobacterium abscessus* complex, comprising subspecies such as *M. abscessus subsp. abscessus,* exemplifies a rapidly expanding NTM and is progressively observed in cystic fibrosis patients. NTM infections can present in diverse forms, ranging from lung disease to disseminated infections, particularly in patients with compromised immune systems [3]. The diagnosis and treatment of NTM infections present significant challenges due to the presence of antibiotic resistance and the absence of a definitive correlation between *in vitro* susceptibility and clinical outcomes [4,6]. The accurate identification of NTM species is imperative for the effective management of these infections, given the diversity of species and their variability in pathogenicity [7].

These species have been particularly linked to the use of mesotherapy, liposuction, and other cosmetic interventions. Due to their presence in aquatic environments and their resistance to multiple drugs, these organisms are often difficult to manage, and they have a wide variety of clinical manifestations, including subcutaneous nodules, abscesses, and fistulas.

Patients infected with NTM typically present with the following comorbidities: Obesity, gastrointestinal diseases, HIV, Cystic Fibrosis (CF), diabetes mellitus, and asthma, respectively [3,8-10]. The diagnosis of these infections is paramount for determining their prognosis and the appropriate utilization of pharmacology. Consequently, the possession of specific cultures, histopathological studies, and molecular tools that facilitate expeditious identification is of the essence. These tools are instrumental in the rapid assessment of antibiotic resistance profiles, a crucial step in preventing future comorbidities and the onset of psychological and aesthetic sequelae in our patients.

Treatment typically necessitates meticulous debridement and the extraction of foreign material. The following drugs are considered to be of use: The following medications are recommended for administration: imipenem (1 g IV every 6 hours), 500 mg of levofloxacin intravenously or orally once daily, 500 mg of clarithromycin orally twice daily, 1 double-strength tablet of Trimethoprim/Sulfamethoxazole (TMP/SMX) orally twice daily, 100 to 200 mg of doxycycline orally once daily, 2 g of cefoxitin IV every 6-8 hours, and 10 to 15 mg/kg of amikacin IV once daily.

It is recommended that combination therapy be administered with at least two drugs that have demonstrated *in vitro* activity. The duration of therapy is 24 months, with the possibility of an extended period if the infected foreign material persists within the body. Amikacin is typically incorporated into the initial 3 to 6 months of treatment. *M. abscessus* and *M. chelonae* are generally resistant to most antibiotics, and they are very difficult or impossible to eradicate. Patients with these infections should be referred to an experienced specialist [11].

Consequently, interdisciplinary efforts among dermatologists, plastic surgeons, and infectious disease specialists are imperative for addressing this issue [12,13].

Materials and methods

A retrospective study was conducted from 2024 to 2025 with the objective of identifying patients who underwent cosmetic procedures and were infected with NTM. The identification of these patients was performed at the species level using the PCR-RFLP technique, as previously described by Talenti et al. in 1999.

The database of a private medical microbiology laboratory in Guadalajara, Jalisco, Mexico, which serves as a reference unit for all such cases, was searched for the demographic data of each patient. The data set encompassed various demographic and clinical characteristics, including gender, age, geographical location, the type of clinical lesion, the affected anatomical region, the cosmetic procedure performed, and the specific mycobacterial species identified through molecular biology.

The data was subsequently entered into an Excel database, and tables and graphs were created to facilitate comprehension.

The confidentiality of the research participants' data was safeguarded in accordance with Article 120 of the General Health Law on Health Research and the Federal Law on the Protection of Personal Data Held by Private Parties. Consequently, the personal data documented in the records was safeguarded by the researchers.

Results

During the study period, a total of 20 patients undergoing cosmetic interventions (17 female and 3 male) were identified as having NTM infections. The diagnosis was performed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique, (Figure 1a & 1b), which has proven to be a highly accurate tool in the field of medical genetics (Smith et al. 2020).

A total of nine distinct mycobacterial species were identified, with *M. chelonae* being the most prevalent, accounting for 11 out of 20 cases (55%), followed by *M. fortuitum*, which was identified in 3 out of 20 cases (15%).

The age of the patients ranged from 17 years to 70 years, with a mean of 43 years, a median of 43, and a mode of 22 and 34 years.

Ziehl-Neelsen staining was performed on all patients, yielding a total of 11 positive results, which constitutes a 55% positivity rate.

A total of seven distinct aesthetic procedures were identified in the patient cohort: Mesotherapy (six cases), lipolytic enzymes (four cases), botulinum toxin (two cases), biostimulators (two cases), tattoos (two cases), liposculpture (three cases), and abdominoplasty (one case).

In the context of the affected topography, nine distinct presentations were identified, with the abdomen exhibiting the highest prevalence, accounting for eight cases (40%), followed by the buttocks and face, which collectively accounted for three cases each (15%).

Three clinical manifestations were observed: Nodules, fistulas, and ulcers. Nodules were the predominant presentation in 11 out of 20 cases (55%), followed by fistulas, which occurred in six cases (30%), and ulcers, which occurred in three cases (15%).

Conclusion

Since the rise in the field of aesthetic medicine we have seen an increasing number of inadequately trained professionals performing these procedures without the minimum knowledge required. This leads to a greater number of iatrogenic complications, particularly infections caused by atypical mycobacteria. These infections have increased proportionally with the rise in clandestine clinics or those lacking the minimum standards required for these procedures.

It is crucial to train healthcare personnel on the management and correct use of available antibiotics to treat these infections,

as well as diagnostic techniques for the proper isolation and identification of the pathogen, such as Ziehl-Neelsen staining, culture in liquid or solid media, and molecular biology techniques. Finally, but no less importantly, we must raise awareness among our patients about the risks involved in undergoing these aesthetic procedures in facilities or with personnel who lack the necessary certifications.

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Supplementary material

No°	gender	age	Ziehl-Neelsen stain	identification method	clinical injury	affected topography	identified species	Associated aesthetic procedure
1	female	70	positive	PCR-RFLP	nodule	abdomen	*M. chelonae*	lipoesculture
2	female	69	positive	PCR-RFLP	FISTULA	glutes	*M. abscessus*	lipolytic enzymes
3	female	64	neagtive	PCR-RFLP	FISTULA	abdomen	*M. fortuitum*	mesoterapy
4	female	60	positive	PCR-RFLP	nodule	abdomen	*M. chelonae*	mesoterapy
5	Male	56	negative	PCR-RFLP	nodule	right arm	*M. fortuitum*	tatto
6	female	56	positive	PCR-RFLP	nodule	abdomen	*M. chelonae*	mesoterapy
7	female	55	positive	PCR-RFLP	FISTULA	lumbar	*M. chelonae ss. Absessus*	lipolytic enzymes
8	female	47	positive	PCR-RFLP	nodule	face	*M. chelonae ss. Absessus*	botulinum toxin
9	female	45	positive	PCR-RFLP	fistula	right submandibular region	*M. chelonae*	biostimulator
10	Male	43	positive	PCR-RFLP	nodule	CHEST	*M. chelonae*	mesoterapy
11	Male	41	negative	PCR-RFLP	FISTULA	abdomen	*M. chelonae*	mesoterapy
12	female	37	negative	PCR-RFLP	sore	thorax, buttocks and abdomen	*M. fortuitum*	lipoesculture
13	female	34	negative	PCR-RFLP	nodule	face	*M. chelonae*	botulinum toxin
14	female	34	positive	PCR-RFLP	FISTULA	abdomen	*M. Chelonae ss. abscessus*	abdominoplasty
15	female	32	positive	PCR-RFLP	FISTULA	abdomen	*M. chelonae*	lipoesculture
16	female	29	negative	PCR-RFLP	sore	glutes	*M. ulcerans*	lipolytic enzymes
17	female	27	negative	PCR-RFLP	nodule	right hand	*M. smegmatis*	tatto
18	female	22	negative	PCR-RFLP	nodule	abdomen	*M. flavescens*	mesoterapy
19	female	22	positive	PCR-RFLP	nodule	face	*M. mucogenicum*	biostimulator
20	female	17	negative	PCR-RFLP	nodule	glutes	*M. gordonae*	lipolytic enzymes

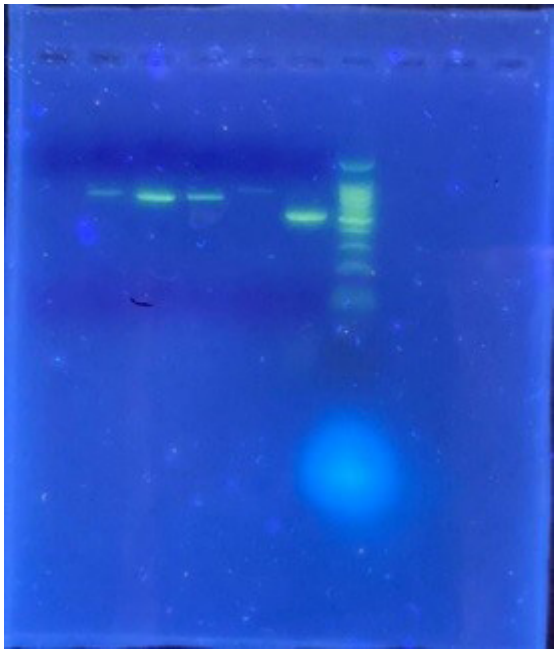

Figure 1: Amplification of PCR products with primers TB11 and TB12 targeting the target gene region of the 65-kilodalton heat shock protein (hsp65) in 1.5% agarose gel.

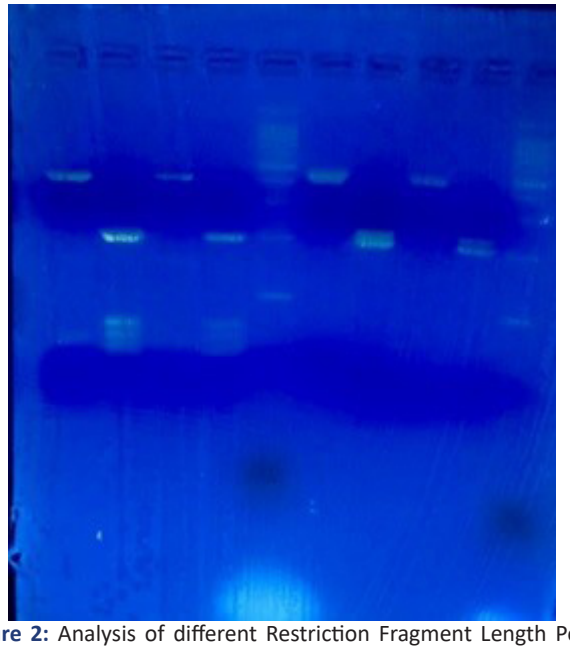

Figure 2: Analysis of different Restriction Fragment Length Polymorphisms (RFLPs) using the restriction enzymes *BstEll* and *HaellI*.