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Case Report

# Isolation by Blood Culture of Non-Tuberculous Mycobacteria and Nocardia in three Immunocompromised Patients

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## Abstract

Three clinical cases are presented in immunocompromised patients, two caused by non-tuberculous mycobacteria (NTM), with positive blood cultures, and one by *Nocardia*, with isolation from pericardial fluid. The patients showed no skin lesions, nor was there evidence of pulmonary lesions. The onset time in two patients was 6 and 40 days, and in a third, 5 months. The latter patient initially presented pulmonary symptoms, but five months passed without progression of the clinical picture, making the source of infection, as in the other two cases, difficult to establish for a oriented clinical diagnosis. The symptoms were insidious, and the diagnosis was delayed in two patients. The rapid, timely, and reliable identification of opportunistic microorganisms allowed for continuation of initial treatment in two patients and modification of it in one. Therefore, all three patients responded well to specific treatment, and the prognosis was favorable in all three cases.

Mass spectrometry (MALDITOF) proved to be a reliable and timely method, identifying two fast-growing NTM species (*Mycobacterium fortuitum* and *Mycobacterium chelonae*), and the species *Nocardia farcinica*, with an acceptance score between 1.91 and 2.2, with high confidence identification in 2 cases (2.0-2.2) and one low confidence (1.91), similar to that found by other authors and reported in the method. The phenotypic characteristics were decisive to first determine the genera and in the case of *Nocardia*, to locate the isolate in the *Nocardia asteroides* complex.

**Kew Words:** immunocompromised patients; nontuberculous mycobacteria; *mycobacterium fortuitum; mycobacterium chelonae; nocardia farcinica*

## Introduction

Non-tuberculous mycobacteria (NTM) and Nocardia species are ubiquitous or cosmopolitan microorganisms found in soil, water, plants and animals [1]. They cause opportunistic infections, most frequently affecting immunocompromised patients [1-3] and occasionally immunocompetent patients [4]. Three clinical forms are observed: localized cases related to skin trauma; disseminated forms with probable primary pulmonary lesions; and related infections due to contamination of medical equipment (catheters and medical devices) [1].

The frequency of these diseases in recent decades has allowed for a better understanding, both in the clinic and in the laboratory [1]. Although these infections are occasional, we must consider the increase in the main chronic degenerative diseases: type 2 diabetes mellitus, cardiovascular diseases, cancer and chronic respiratory diseases, which indirectly condition a compromise in the cellular immunity of pediatric and geriatric patients [5].

The combination of an accurate clinical diagnosis, appropriate clinical sample collection, and immediate processing or referral to a reference laboratory is crucial for establishing a timely and reliable diagnosis and establishing specific treatment in these patients [6].

The purpose of this paper is to present three clinical cases in immunocompromised patients, who present a challenge for laboratory diagnosis in the reliable isolation and identification of these opportunistic microorganisms.

## Clinical cases.

| | Case 1 | Case 2 | Case 3 |
|---|---|---|---|
| Key and origin | MIC-22-25. LEE. LESP* Chiapas | MIC-62-25.GHD. LESP* Hidalgo | MIC-92-25. FLQ. LESP* Michoacán |
| Age: | 17 y | 14 y | 3.9 y |
| Sex | F | M | F |
| Baseline diagnosis. | Hypertrophic cardiomyopathy | Acute lymphoblastic leukemia | Acute lymphoblastic leukemia B 8 |
| Start date | 14-02-2025 | 6-05-2025 | 17-02-2025 |
| Date of collection and clinical sample | 02-20-2025.Pericardial fluid | 15-06-2025. Blood culture | 16-07-2025 Blood culture |
| Species identified in the LESP (*) | *Nocardia* | *Nocardia* | Isolation: acid-fast bacilli |
| Species identified in the InDRE, with the MALDI-TOF value | *Mycobacterium fortuitum*. 2.21 | *Nocardia farcinica*. 2.0 | *Mycobacterium chelonae*. 1.91 |

**Table 1:** shows the general data of the patients.

(*) State Public Health Laboratory. (National Network State Public Health Laboratories)

## Case 1

A 17-year-old patient was diagnosed with hypertrophic cardiomyopathy secondary to laminar pericardial effusion, with no compromised ventricular function. CKD stage 5 (chronic kidney disease stage 5) was receiving renal replacement therapy with hemodialysis. CLABSI (central line-associated bloodstream infection) due to *Staphylococcus epidermidis* was diagnosed. The patient's onset was on February 14, 2025, and a pericardial fluid sample was taken on February 20, 2025. The culture was positive, the bacilloscopy was positive, and the morphology was characteristic of *Nocardia*. Treatment started with trimethoprim-sulfamethoxazole.

The culture was sent to the InDRE (Institute of Epidemiological Diagnosis and Reference, Coordinator of the National Network of State Laboratories, which participates in receiving clinical cases and strains for a reliable and timely diagnosis of opportunistic mycobacteria). The strain was received in a blood culture bottle and reseeded in blood agar, MacConkey agar, and Sabouraud dextrose agar media with chloramphenicol. The plates were incubated at 30 and 37°C. The plates showed a fast-growing strain, with frank development between 48 and 72 hours. Microscopic examination, as well as morphological characteristics, did not correspond to the genus *Nocardia*, directing the culture to the genus *Mycobacterium*. The strain was identified by the automated Bruker Daltonik MALDI Biotyper, with a value of 2.0, which grants a high confidence evaluation, compatible with *Mycobacterium fortuitum*. Treatment with trimethoprim-sulfamethoxazole was replaced by specific treatment for *Mycobacterium fortuitum*, and the patient responded very well. The patient currently continues her renal dialysis program, without any complications from opportunistic infections. Figure 1 shows cultures and microscopic images of *Mycobacterium fortuitum*.

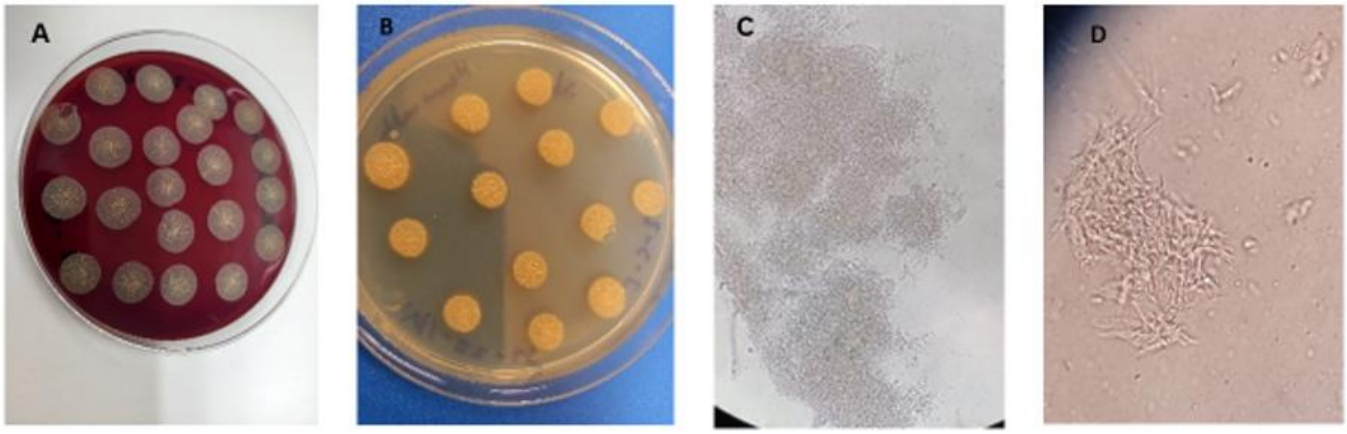


**Figure 1**: Cultures and microscopic images of *Mycobacterium fortuitum*. **A**. Reseeding of pericardial fluid in blood culture on blood agar at 30 ºC for 72 h. **B**. Culture on Sabouraud dextrose agar with chloramphenicol added at 30 ºC for 72 h. **C**. Microscopic image, 40X, showing bacilli forming microcolonies. **D**. Microscopic image, 100x, showing individual long bacilli, attached, without longitudinal union and without branching.

## Case 2.

A 14-year-old male was diagnosed with acute lymphoblastic leukemia in November 2022 and is currently in week 106 of maintenance. He began on May 6, 2025, with gastro-alimentary vomiting, progressively increasing to up to 10 times per day, followed by headache, vertigo, and ataxia. Plain and contrast-enhanced MRI studies revealed a brain abscess in the cerebellar vermis, with perilesional edema and partial obliteration of the fourth ventricle. On June 15, 2025, a median suboccipital craniotomy was performed, with drainage of purulent material, which was sent for histopathological studies and culture. Histopathology results reported a morphology compatible with *Nocardia*. Cultures revealed growth in the blood culture bottle and thioglycolate broth. The strain was sent to the InDRE for identification. The strain was received in a blood culture bottle and replated on blood agar, MacConkey agar, and Sabouraud dextrose agar media with chloramphenicol. The plates were incubated at 30 and 37°C. The plates exhibited slow-growing, rough, yellow-orange colonies. The culture revealed a microscopic image of branched, microsiphonated filaments typical of the genus *Nocardia*. The strain hydrolyzed urea and was casein negative, placing it in the *Nocardia asteroides* complex. The strain was identified by the Bruker automated

MALDI-TOF system with a score of 2.0, which gives a high confidence rating, compatible with *Nocardia farcinica*. The patient showed a good response to the therapeutic combination of meropenem and linezolid and is currently recovering progressively with treatment. Figure 2 shows cultures and microscopic images of *Nocardia farcinica*.

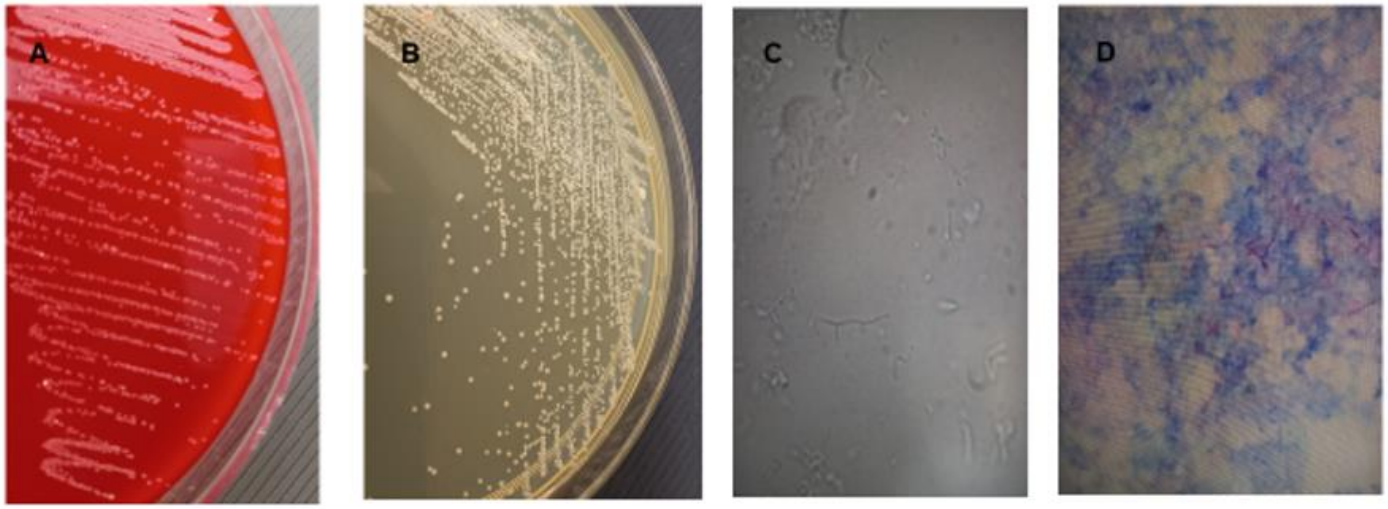


**Figure 2:** Cultures and microscopic images of *Nocardia farcinica*. **A**. Reseeding of purulent material from a brain abscess on blood agar, in blood culture, at 30 ºC, for 72 h. **B**. Culture on Sabouraud dextrose agar with chloramphenicol added, at 30 ºC, for 72 h. **C**. Fresh microscopic image 100X, showing longitudinally attached and branched cells. **D**. Ziheel Neelsen staining, 100x, showing individual acid-fast cells, with the appearance of branched bacilli.

## Case 3.

A 3.9-year-old female patient was diagnosed with B-cell acute lymphoblastic leukemia on March 5, 2025, and received 3 cycles of oncologic treatment, starting on March 10, 2025. The patient was diagnosed with moderate to severe malnutrition. Weight 11 kg. Onset 02/17/25, dry cough, immunocompromised patient. Sample taken 07/16/25. Central/peripheral blood culture in June 25 reported isolation revealing acid-fast bacilli. The culture was sent to the InDRE for taxonomic confirmation. The strain was received on Lowenstein-Jensen medium. The culture was replated on blood agar, MacConkey agar, and Sabouraud dextrose agar media, with the addition of chloramphenicol. The plates were incubated at 30 and 37°C. The plates showed a rapidly growing strain, with frank development at 72 hours. Microscopic examination, as well as morphological characteristics, ruled out the genus *Nocardia*, directing the culture to the genus *Mycobacterium*. Determination confirmed by InDRE on 20-8-25 as *Mycobacterium chelonae*, with a MALDI-TOF value of 1.91 and a low-confidence identification interpretation. Antimicrobial treatment was started on 20-8-25: clarithromycin 15 mg/kg/day, PO every 12 hours (12 months); amikacin 15 mg/kg/day, *iv* every 12 hours (8 weeks); linezolid (30 mg/kg/day), PO every 12 hours (12 months).

Current diagnosis: B-cell acute lymphoblastic leukemia, undergoing chemotherapy (fourth cycle ongoing), plus severe malnutrition, plus disseminated *Mycobacterium chelonae* infection, plus previous hepatosplenic and renal fungomas, persistent lesions on ultrasound (USG), plus gallstones, plus moderate normocytic anemia.

Plan and management: Continue chemotherapy regimen, complete extended course of antibiotics, close monitoring of kidney, liver, and hearing function, close surveillance for fever, signs of chemotoxicity, hematologic complications, and intensive nutritional support. The prognosis is guarded, given the underlying neoplasia, severe opportunistic infection, and nutritional status. Figure 3 shows cultures and microscopic images of *Mycobacterium chelonae*.

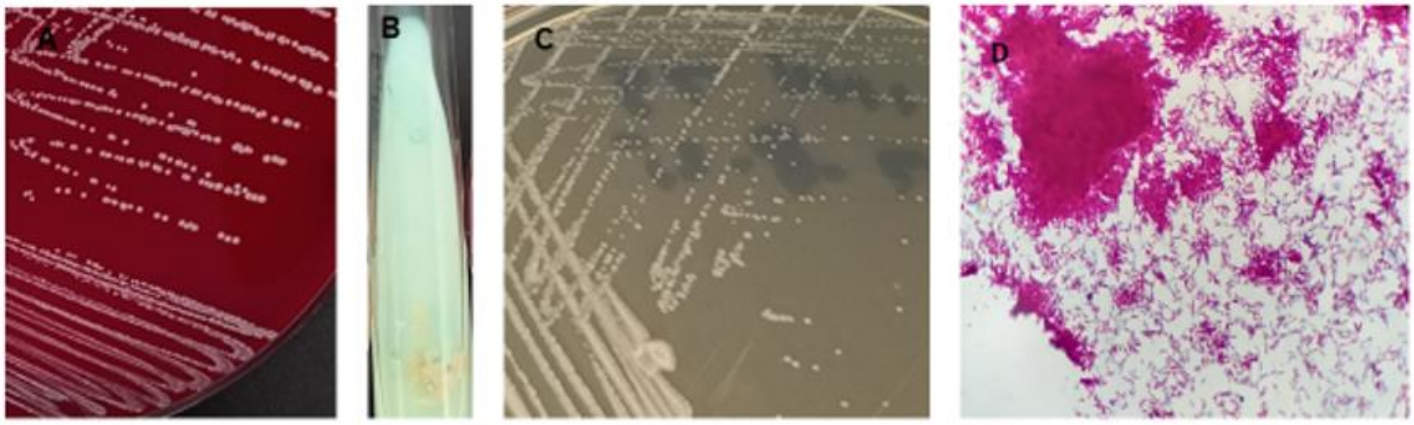


**Figure 3**: Cultures and microscopic images of *Mycobacterium chelone*. **A**. Culture on blood agar, from blood culture, at 30°C, for 72 h. **B**. Culture on Lowenstein-Jensen stain at 37 °C, for 72 h. **C**. Culture on Sabouraud dextrose agar with chloramphenicol added, at 30 °C, for 72 h. D. Microscopic image, using Ziehel-Neelsen staining, 100x, showing individual acid-fast cells, resembling branched bacilli.

## Discussion

These are immunocompromised patients with chronic degenerative diseases and probable coexistence of other infections, which implies a challenge in their clinical and laboratory management (7). None of the 3 patients had skin lesions or a history of trauma, nor did they present pulmonary symptoms. The patient diagnosed with hypertrophic cardiomyopathy, secondary to laminar pericardial effusion, had a *Staphylococcus epidermidis* infection, associated with the central line. The appearance of a sudden acute picture, in a period of 6 days, with pericardial effusion, led opportunely to the puncture of the pericardial fluid, whose bacteriological study, revealed a positive culture and the presence of acid-fast bacilli, with characteristic morphology of *Nocardia*, diagnosed by histopathological examination. The strain obtained was sent to a Reference Laboratory (InDRE), where it was identified as *Mycobacterium fortuitum*, in the automated MALDI-TOF equipment (3, 8), which allowed to modify the antibiotic regimen and establish a specific treatment, for the benefit of the patient and changing the prognosis of the disease.

The patient with brain abscess presented with vomiting, followed by headache and vertigo. Imaging studies revealed brain abscess, which was drained 45 days after the onset of clinical symptoms. The histopathological diagnosis was immediate, and the result was compatible with a *Nocardia* infection. The cultures were sent to the InDRE for taxonomic confirmation. Mass spectrometry is a method with good results for the identification of *Nocardia* species (3,8). The Bruker MBT Compass Library Revision H includes 89 *Nocardia* species in its database. Apparently, cultures that develop within 24 to 48 hours have low sensitivity for their identification. Timely and reliable identification of Nocardia species is important to expedite antimicrobial treatment and establish a good prognosis for patients (3). The identified species, *Nocardia farcinica* (2-3; 6-9), is one of the species of the *Nocardia asteroides* complex and is one of the most common, causing human infections, especially in patients with hematological cancers (10).

In the case of *Mycobacterium chelonae* infections (8, 10), this species usually occurs in immunosuppressed patients (9, 10, 11), but cases have been described only in immunocompetent patients, associated with trauma (8). Patients with hematological-oncological diseases constitute a high-risk group (9), of difficult treatment (10). A strong association between NTM infections and patients with acute lymphoblastic leukemia (ALL) has been suggested (9), considering the latter as a high-risk group. The species *Mycobacterium fortuitum* and *Mycobacterium chelonae* are among the most frequent [9].

In all three cases, Ziehl-Neelsen staining revealed the presence of acid-fast bacilli by microscopic examination. In the patient with brain abscess, the results were conclusive, and the diagnosis was categorical: *Nocardia* infection. Culture was also key, showing slow-growing, dry, rough, orange-yellow colonies typical of *Nocardia*. In the other two cases, there were marked differences; growth in culture media was rapid, taking between 48 and 72 hours. *Mycobacterium fortuitum* and *Mycobacterium chelonae* are fast-growing species [4,11; 12-13]. In our experience, clinical isolates from patients grow very well on blood agar and Sabouraud dextrose agar, although there are reports where isolates have developed slowly, taking between 7 and 9 days (9). It should be noted that fresh microscopic observations are very important, as they revealed bacterial clusters that mimic *Nocardia* characteristics, especially in the coloring, but the long, single, branched filaments, as is the case with *Nocardia*, are not observed. This phenotypic characteristic is essential for differentiating fast-growing NTM from *Nocardia* species (1).

Although NTM and *Nocardia* bacteremias are rare, and even though blood culture has a low recovery of these pathogens (14-17), this test continues to provide great utility in the diagnosis of infectious diseases, both bacterial and fungal pathogens. Since clinical manifestations are not specific in nocardiasis and NTM infections (4, 5, 10, 13, 14), differential diagnosis with pathogenic fungi is very important, since blood culture has allowed to obtain very useful results in infections by *Candida* species, *Histoplasma capsulatum*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Fusarium verticillioides* and less frequent species such as *Trichosporon ashaii* and *Rhodotorula glutinis*, as confirmed by other authors (6, 11). Of the three opportunistic species isolated, two strains identified (*Mycobacterium fortuitum*, *Nocardia farcinica*), had a record of 2.21 and 2.0, corresponding to high confidence evaluations (+++), while *Mycobacterium chelonae* had a record of 1.91, considered as a low confidence identification (+), however, there is only a difference of 0.09 hundredths, with the value of 2.0, which grants a high confidence evaluation. In this work, the combination of phenotypic tests and the use of automated tests (MALDI-TOF), allowed a rapid identification of clinical isolates. The results are reliable and like those obtained by other researchers (13).

## Conclusions And Recommendations

Three clinical cases are presented in immunocompromised patients, two caused by nontuberculous mycobacteria (NTM), with positive blood cultures, and one by *Nocardia*, with isolation from pericardial fluid. The patients did not present skin lesions, nor was there evidence of lung lesions. In the diagnosis of infections due to rapidly growing mycobacteria and *Nocardia* species, the presence of acid-fast bacilli in the blood culture and the phenotypic characteristics in the culture media are decisive for laboratory diagnosis. When a sterile clinical sample (blood culture) is analyzed and cultured, a rapid diagnosis is possible with microscopic examination and timely reseeding in various culture media.

Molecular testing is the gold standard for the identification of NTM and *Nocardia* species. In this study, the combination of phenotypic testing and the use of automated assays (MALDI-TOF) allowed for rapid and timely identification of clinical isolates of three opportunistic species: *Mycobacterium fortuitum*, *Mycobacterium chelonae*, and *Nocardia farcinica*.

Although the symptoms were insidious and diagnosis was delayed in two cases, all three responded well to specific treatments, and the prognosis was favorable in all three patients.

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