ACCESS MICROBIOLOGY

CASE REPORT

Morgado *et al.*, *Access Microbiology* 2022;4:000317

DOI 10.1099/acmi.0.000317

MICROBIOLOGY SOCIETY

OPEN ACCESS

Multidrug-resistant *Mycolicibacterium fortuitum* infection in a companion cat (*Felis silvestris catus*) in Brazil

Sergio Morgado[1,*], Nilcéia de Veiga Ramos[2], Bárbara Bianca do Nascimento Pereira[3], Fernanda Freitas[1], Érica Lourenço da Fonseca[1] and Ana Carolina Vicente[1,*]

Abstract

Mycolicibacterium fortuitum is a fast-growing bacterium and an opportunistic pathogen implicated in human and animal infections. Here we report the first case and genetic characterization of a strain of *M. fortuitum* isolated from skin lesions of a companion cat with atypical cutaneous mycobacteriosis in Brazil. In addition, the genome of this strain was sequenced, representing the first genome of this opportunistic pathogen isolated from an animal infection. The *in silico* and *in vitro* analysis regarding antibiotic resistance of this strain showed an intrinsic multiresistance antibiotic profile. However, this strain showed sensitivity to amikacin and ciprofloxacin, and the cat was treated long-term with these drugs.

INTRODUCTION

Mycolicibacterium fortuitum (also known as *Mycobacterium fortuitum*) is a fast-growing non-tuberculous mycobacteria (NTM) that is saprophytic and ubiquitous in the environment (found in soil and water), being recognized as a human and animal pathogen worldwide [1–6]. This species has been isolated from a variety of animals, including birds, reptiles, fish, amphibians, invertebrates, wild boar, cattle, seals and armadillos, in addition to domestic animals such as dogs and cats [7]. There are reports from different parts of the world of mycobacteriosis in cats indicating a prevalence of *M. fortuitum* as the etiologic agent. Usually, cats present skin infections in the form of non-healing ulcerative pyogranulomatous dermatitis and panniculitis [7–11]. In other animals, such as cattle, *M. fortuitum* can cause chronic mastitis [12]. In addition, infections caused by *M. fortuitum* are characterized by high resistance to antibiotics, such as aminoglycosides, beta-lactams, macrolides, rifamycins, and tetracyclines [13–15]. Recently, a genomic study revealed that a set of genes associated with resistance to these antibiotics (a*ph(3")-Ic*, *aac(2')-Ib*, *arr*-1, *bla*F, *erm*39, *rbp*A, *rox*, and *tap*) appears to be ubiquitous in *M. fortuitum* species [16]. Since treatment of mycobacteriosis is long-term, isolation and antibiotic susceptibility assays are recommended to avoid delays in treatment [9–11]. Here we present the first reported case of a companion cat (*Felis silvestris catus*) with mycobacteriosis caused by *M. fortuitum* in Brazil.

CASE REPORT

An approximately 6-year-old male domestic cat with access to the outdoors (4.8 kg body weight) presented in July 2020 for examination of skin nodules in the lower abdomen, near the right pelvic limb and inguinal region. The cat had been adopted by the owner in 2016, and its exact age and history prior to adoption were unknown. Previously, the cat's owner had consulted other veterinarians, who performed a biopsy and histopathologic examination suggestive of pyogranulomatous panniculitis. The cat was treated with anti-inflammatory steroids and antimicrobial medications such as amoxicillin with clavulanic acid, sulfamethoxazole with trimethoprim (systemic), and polymyxin B (topical) without success. So, the owner visited a veterinary centre specialising in cats, where new tests were performed. The lesions were firm and painful and had been noticed by the owner about ten months earlier. The masses infiltrated the soft tissue of the right lower abdomen and inguinal region, where they exhibited fistula-like

Received 02 September 2021; Accepted 09 December 2021; Published 28 February 2022

Author affiliations: [1]Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Genética Molecular de Microrganismos, Rio de Janeiro, Brazil; [2]Universidade de Vassouras, Faculdade de Ciências Médicas de Maricá, Rio de Janeiro, Brazil; [3]Clinicat, Rio de Janeiro, Brazil.

***Correspondence:** Sergio Morgado, sergio.morgado@ioc.fiocruz.br; Ana Carolina Vicente, anapaulo@ioc.fiocruz.br

Keywords: Zoonosis; micobacteria; genome; pathogen; animal; veterinary.

Abbreviations: FeLV, feline leukaemia virus; FIV, feline immunodeficiency virus; NTM, non-tuberculous mycobacteria; TSA, trypticase soy agar.

The draft genome sequence (JAEQRQ000000000) and the raw sequence reads (SRR15257947) are publicly available on the NCBI under BioProject PRJNA691870.

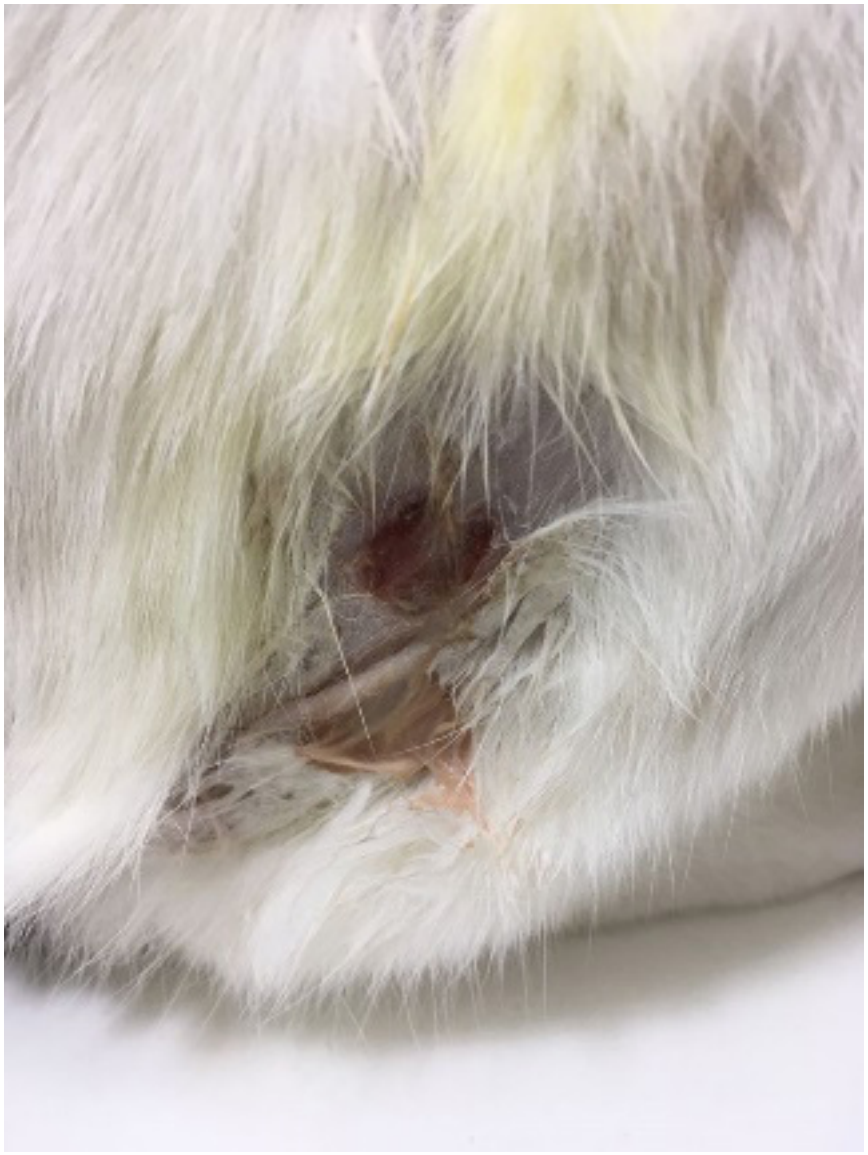

Fig. 1. The suppurative and ulcerative aspects of the lesion near the right pelvic limb of the cat.

tracts (Figs. 1 and 2). On physical examination, the cat was in good general condition and no other abnormalities were noted. Haematology, blood biochemistry, and electrolytes showed lymphopenia (1340 cells μl^{-1}; reference interval 1500–7000 cells μl^{-1}) and an increase in serum globulin concentration (6 g dl^{-1}; reference interval 2.5–5.1 g dl^{-1}). Tests for feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) were negative, according to the owner. Tissue samples were collected with a 5 mm punch and sent for repeat histopathologic analysis and bacterial isolation. Histopathologic examination revealed a lesion pattern involving a large portion of the dermis and extending to the adipose panniculus and subcutaneous muscle tissue with a severe inflammatory reaction. This inflammation was characterised by proliferation of mature fibrous connective tissue, oedema, and poorly demarcated nodular inflammatory infiltrates in a coalescing multifocal pattern composed mainly of neutrophils. The findings were suggestive of atypical cutaneous mycobacteriosis. Due to the failure of previous treatment with anti-inflammatories and some antimicrobial drugs, in addition to the suspicion of mycobacteriosis, we performed bacterial isolation and antibiotic susceptibility testing. Bacterial isolation was performed in trypticase soy agar (TSA) culture medium supplemented with 0.05% Tween-80 (Sigma-Aldrich) at 22 °C for 72 h. Taxonomic identification was based on the Multilocus Sequence Analysis scheme with amplification and sequencing of 16S rRNA, *hsp*65 and *rpo*B genes [17], indicating infection by *Mycolicibacterium fortuitum* (named 7G strain). *In vitro* antibiotic susceptibility assays of the *M. fortuitum* 7G strain showed a high rate of resistance to various drug classes based on E-test, with the following MICs: Azithromycin ≥256 μg ml^{-1} and Clarithromycin ≥32 μg ml^{-1} (macrolides); Streptomycin ≥32 μg ml^{-1} and Tobramycin ≥32 μg ml^{-1} (aminoglycosides); Meropenem ≥32 μg ml^{-1} (carbapenem); Cefalotin

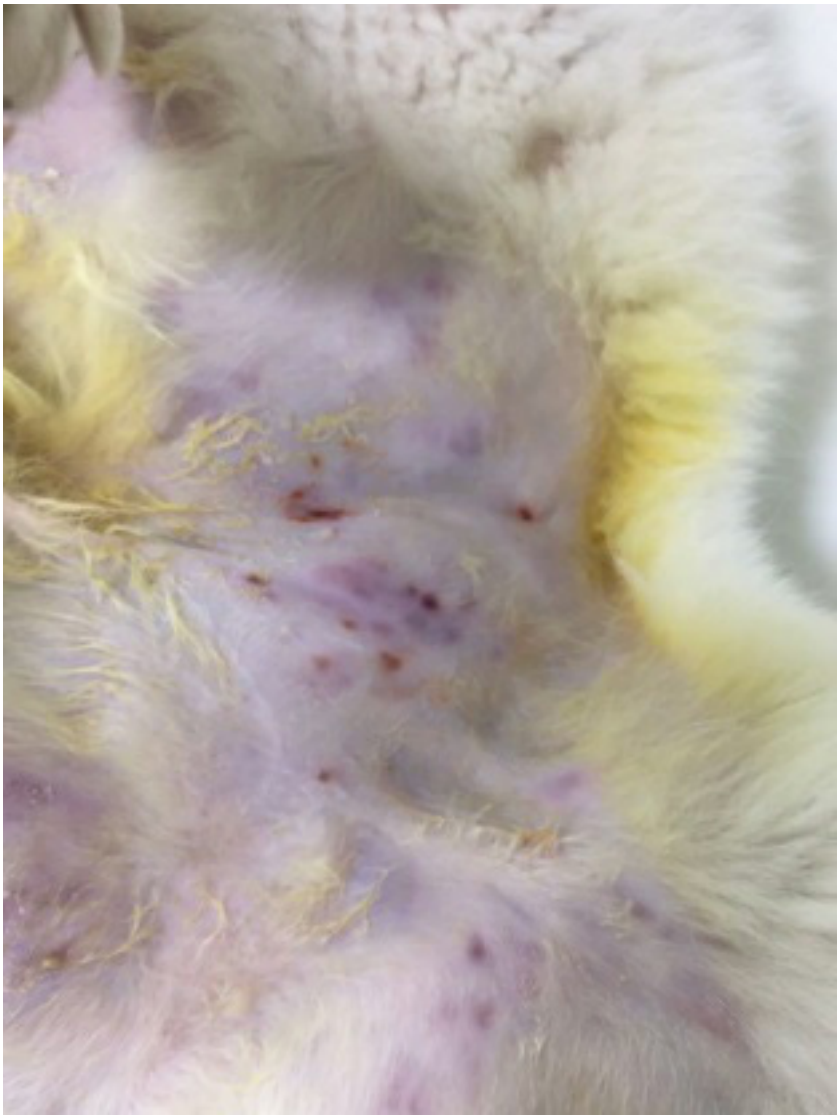

Fig. 2. Multiple subcutaneous nodules in the inguinal region of the cat.

≥256 μg ml^{-1} and Cefepime ≥256 μg ml^{-1} (cephalosporins); and Rifampicin ≥32 μg ml^{-1} (rifamycin). Other drugs were also verified by disc diffusion assays (Table 1). Despite the extended antibiotic resistance profile, this strain showed sensitivity to amikacin (aminoglycoside) and ciprofloxacin (quinolone). Therefore, the cat was treated long-term with pradofloxacin (5 mg/kg), an alternative quinolone, because the bioavailability of ciprofloxacin in cats is low.

To gain additional insight into the presence of other antibiotic resistance determinants in this *M. fortuitum* 7G strain, we performed sequencing of its genome. Genomic DNA was extracted (100 ng) using NucleoSpin Microbial DNA (Macherey-Nagel) and sequenced using the Nextera XT library preparation kit on the Illumina HiSeq 2500 platform with 2×250 bp paired-end reads. Genomic analysis using Comprehensive Antibiotic Resistance Database v.3.1.3 [18] revealed the presence of several antibiotic resistance genes, such as *aph*(3")-Ic (aminoglycoside resistance), *arr*-1 (rifamycin), *bla*F (β-lactamase), *erm*39 (macrolide), *aac*(2')-Ib (aminoglycoside), and *rbp*A (rifamycin). In addition, we identified four secretion systems of the VII type (ESX-1, ESX-3, ESX-4, and ESX-4-bis) that have previously been associated with virulence and survival in *Mycobacterium* [19].

DISCUSSION

Cases of mycobacterial disease associated with non-tuberculous mycobacteria are increasing worldwide in humans and animals [1, 5]. Companion animals, especially cats and dogs, that are susceptible to these infections may pose a public health risk. In addition, mycobacteriosis can be difficult to diagnose and lengthy to treat [9, 11, 20]. A common aetiological agent in mycobacterial

Table 1. Antibiotic susceptibility profile based on disk diffusion

Antibiotic class	Antibiotic	Resistance
Aminoglycosides	Amikacin, Gentamicin, Kanamycin, Neomycin	S
	Streptomycin, Tobramycin	R
Carbapenems	Ertapenem, Imipenem, Meropenem	R
Cephalosporins 1Gen	Cephalotin	R
Cephalosporins 2Gen	Cefoxitin	S
Cephalosporins 3Gen	Cefotaxime, Ceftriaxone	R
Cephalosporins 4Gen	Cefpirome	R
Macrolides	Azithromycin, Clarithromycin, Spectinomycin	R
Penicillins	Amoxicillin, Ampicillin-Sulbactam, Carbenicillin, Oxacillin, Penicillin G, Piperacillin-tazobactam	R
Quinolones	Nalidixic Acid	R
	Ciprofloxacin, Ofloxacin, Levofloxacin, Norfloxacin	S
Sulfonamides	Sulfametaxazole, Sulfametaxazole-Trimetroprime	S
	Trimetroprime	R
Tetracyclines	Minocycline, Tetracycline	R
Monobactams	Aztreonam	R
Glycopeptide	Vancomycin	R
Lincosamide	Clindamycin, Lincomycin	R
Others	Chloramphenicol, Fosfomycin, Nitrofurantoin, Rifampicin, Ticarcillin-clavulanic	R
	Tigecycline	S

S, susceptible; R, resistant.

disease is *Mycolicibacterium fortuitum*, an organism ubiquitous in the environment with a typical clinical presentation in skin and soft tissue [21]. Therefore, the identification of a multidrug-resistant strain of *M. fortuitum* causing disease in a companion cat in Brazil demonstrates the importance of proper diagnosis and treatment of animal diseases for the one health. Although histopathological examination is a reliable method for diagnosing cutaneous mycobacteriosis, without culture and molecular analysis, the aetiological agent of the infection could not have been diagnosed as *M. fortuitum*. Furthermore, proper antibiotic treatment is crucial in this matter as it has an impact on the prompt recovery of the animal and the presence of this pathogen in this environment. Here, the antibiotic susceptibility profile of the 7G strain was determined *in vitro* using disc diffusion and E-test to provide appropriate antibiotic therapy. Interestingly, this strain showed resistance to rifampicin, which is worrying as this drug is used worldwide as first-line therapy for human tuberculosis [22]. In addition, our study provided the first genome of an *M. fortuitum* strain associated with animal disease. Through genomic analysis, we identified several genes on the chromosome associated with antibiotic resistance, suggesting that they are inherent to this organism and not related to plasmid-mediated acquisition. Indeed, in a previous *in silico* study of *Mycolicibacterium* plasmids, no notable antibiotic resistance genes were observed in plasmids of *M. fortuitum* [23]. Another aspect verified in the genome of the 7G strain was the type VII, present in four loci and reported to be associated with virulence, iron uptake and conjugation in several species of the *Mycobacteriaceae* family [24]. Thus, these systems could favour the adaptation and colonisation of the *M. fortuitum* strain in different environments.

In conclusion, culture and molecular analysis of mycobacterial infections accelerate diagnosis and allow appropriate treatment and control of the aetiological agent.

Funding information
This study was partially supported by the postdoctoral fellowship Inova Fiocruz/Fundação Oswaldo Cruz.

Author contributions
Conception, design, data analysis, and manuscript writing: S.M.M., E.L.F., A.C.P.V., N.V.R.; experimental work: F.F.J., N.V.R., B.B.N.P.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
As this study involved samples collected in the routine of the veterinary clinic, being considered the veterinary treatment that the animal needed, it was exempt from ethical approval according to the guidelines of the Research Animal Ethics Committee of the Oswaldo Cruz Institute (Normative Resolution No. 23, of July 23, 2015). In addition, the veterinarians who attended the animal and sent the samples for analysis were also co-authors of the study. The photos were taken with the permission of the cat's owner.

References

1. Halstrom S, Price P, Thomson R. Review: Environmental mycobacteria as a cause of human infection. *Int J Mycobacteriol* 2015;4:81–91.
2. Barandiaran S, Martínez Vivot M, Falzoni E, Marfil MJ, Pérez Tort G, *et al*. Mycobacterioses in dogs and cats from Buenos Aires, Argentina. *J Vet Diagn Invest* 2017;29:729–732.
3. Delghandi MR, El-Matbouli M, Menanteau-Ledouble S. Mycobacteriosis and infections with non-tuberculous mycobacteria in aquatic organisms: a review. *Microorganisms* 2020;8:E1368.
4. Shrivastava K, Kumar C, Singh A, Narang A, Giri A, *et al*. An overview of pulmonary infections due to rapidly growing mycobacteria in South Asia and impressions from a subtropical region. *Int J Mycobacteriol* 2020;9:62–70.
5. Winburn B, Sharman T. Atypical mycobacterial disease. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing, 2021.
6. Mugetti D, Tomasoni M, Pastorino P, Esposito G, Menconi V, *et al*. Gene sequencing and phylogenetic analysis: powerful tools for an improved diagnosis of fish mycobacteriosis caused by *Mycobacterium fortuitum* group members. *Microorganisms* 2021;9:797.
7. Couto SS, Artacho CA. *Mycobacterium fortuitum* pneumonia in a cat and the role of lipid in the pathogenesis of atypical mycobacterial infections. *Vet Pathol* 2007;44:543–546.
8. Woerde DJ, Martin PA, Govendir M. Susceptibility of rapidly growing mycobacteria isolated from Australian cats to ivermectin, moxidectin, ceftiofur and florfenicol. *J Feline Med Surg* 2015;17:1065–1068.
9. Krajewska-Wędzina M, Dąbrowska A, Augustynowicz-Kopeć E, Weiner M, Szulowski K. Nontuberculous mycobacterial skin disease in cat; diagnosis and treatment - Case report. *Ann Agric Environ Med* 2019;26:511–513.
10. Munro MJL, Byrne BA, Sykes JE. Feline mycobacterial disease in northern California: Epidemiology, clinical features, and antimicrobial susceptibility. *J Vet Intern Med* 2021;35:273–283.
11. Apostolopoulos N, Prenger-Berninghoff E, Wildermuth B, Moser I, Hillemann D, *et al*. Mycobacterium setense isolated from a cat with atypical mycobacterial panniculitis. *Tierarztl Prax Ausg K Kleintiere Heimtiere* 2021;49:390–396.
12. Bercovier H, Vincent V. Mycobacterial infections in domestic and wild animals due to *Mycobacterium marinum*, *M. fortuitum*, *M. chelonae*, *M. porcinum*, *M. farcinogenes*, *M. smegmatis*, *M. scrofulaceum*, *M. xenopi*, *M. kansasii*, *M. simiae* and *M. genavense*. *Rev Sci Tech* 2001;20:265–290.
13. Santos DR de S, Lourenço MCS, Coelho FS, Mello FCQ, Duarte RS. Resistance profile of strains of *Mycobacterium fortuitum* isolated from clinical specimens. *J Bras Pneumol* 2016;42:299–301.
14. Lin C, Russell C, Soll B, Chow D, Bamrah S, *et al*. Increasing prevalence of nontuberculous mycobacteria in respiratory specimens from US-Affiliated Pacific Island Jurisdictions1. *Emerg Infect Dis* 2018;24:485–491.
15. Shen Y, Wang X, Jin J, Wu J, Zhang X, *et al*. *In vitro* susceptibility of *Mycobacterium abscessus* and *Mycobacterium fortuitum* isolates to 30 antibiotics. *Biomed Res Int* 2018;2018:4902941.
16. Morgado SM, Ramos NV, Freitas F, Fonseca EL, Vicente AC. *Mycolicibacterium fortuitum* genomic epidemiology, resistome and virulome. *Mem Inst Oswaldo Cruz* 2021;116:11.
17. Kim SH, Shin JH. Identification of nontuberculous mycobacteria using multilocous sequence analysis of 16S rRNA, hsp65, and rpoB. *J Clin Lab Anal* 2018;32.
18. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, *et al*. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 2017;45:D566–D573.
19. Lagune M, Petit C, Sotomayor FV, Johansen MD, Beckham KSH, *et al*. Conserved and specialized functions of Type VII secretion systems in non-tuberculous mycobacteria. *Microbiology (Reading)* 2021;167.
20. Silva DLF da, Valandro LDS, Velho PENF, França AFE da C. Atypical cutaneous mycobacteriosis caused by *M. fortuitum* acquired in domestic environment. *An Bras Dermatol* 2020;95:390–391.
21. da Silva Neto JP, Machado KNC, Roisman L. Choroidal granuloma caused by *Mycobacterium fortuitum*. *Int J Retina Vitreous* 2019;5:37.
22. Beloor Suresh A, Rosani A, Wadhwa R. Rifampin. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing, 2021.
23. Morgado SM, Vicente ACP. Comprehensive in silico survey of the Mycolicibacterium mobilome reveals an as yet underexplored diversity. *Microb Genom* 2021;7:000533.
24. Mortimer TD, Weber AM, Pepperell CS. Evolutionary thrift: mycobacteria repurpose plasmid diversity during adaptation of Type VII secretion systems. *Genome Biol Evol* 2017;9:398–413.