New Microbiologica, 39, 2, 156-159, 2016, ISN 1121-7138

SHORT COMMUNICATION

Madura foot in Europe: diagnosis of an autochthonous case by molecular approach and review of the literature

Jessica Mencarini[1], Alberto Antonelli[2,3], Guido Scoccianti[4], Laura Bartolini[5], Giuliana Roselli[6], Rodolfo Capanna[4], Gian Maria Rossolini[2,3,5], Alessandro Bartoloni[1,7], Filippo Bartalesi[7]

[1]*Infectious Diseases Unit, Department of Experimental and Clinical Medicine, University of Florence, Italy;*
[2]*Department of Medical Biotechnologies, University of Siena, Italy;*
[3]*Department of Experimental and Clinical Medicine, University of Florence, Italy;*
[4]*Orthopaedic Oncology Unit, Careggi Hospital, Florence, Italy;*
[5]*Microbiology and Virology Unit, Careggi Hospital, Florence, Italy;*
[6]*Department of Diagnostic Imaging, Careggi Hospital, Florence, Italy;*
[7]*Infectious and Tropical Diseases Unit, Careggi Hospital, Florence, Italy*

SUMMARY

Madura foot is a chronic granulomatous infection of the soft-tissue of the foot and it is endemic in tropical and subtropical countries. Some cases have also been reported in local people or migrants in temperate countries. The microbiological diagnosis requires prolonged bacterial cultures in aerobic and anaerobic conditions, but the use of the molecular approach could be helpful for an early and rapid diagnosis.

We describe an autochthonous case of *Actinomadura madurae* foot infection in an Italian woman. The diagnosis was achieved 36 months after symptoms onset by PCR detection and sequencing of 16S rDNA directly on biopsy. She started therapy with rifampin, trimethoprim-sulfamethoxazole, and amikacin. After 3 months the pain had disappeared and the swelling subsided.

We reviewed the literature on Madura foot due to bacterial causative agents in Europe and observed that the median time from onset to diagnosis is high, possibly due to several factors like the difficulties of the microbiological and radiological diagnosis. Our case report and the review of literature point out that the implementation of a surveillance system, the involvement of an infectious diseases specialist, with experience in tropical diseases, and the availability of a microbiology unit to perform feasible and rapid molecular diagnostic tests could result in an earlier diagnosis and an optimal antibiotic therapy of this rare but difficult-to-treat and, above all, difficult-to-diagnose infection.

Received December 12, 2015 *Accepted* April 4, 2016

INTRODUCTION

Mycetoma is a chronic granulomatous infection of the soft-tissue, due to fungi (Eumycetoma) or bacteria (Actinomycetoma). The infection is endemic in tropical and subtropical countries, but some cases have also been reported in local people or migrants in temperate countries, including some European countries (Albania, Bulgaria, Greece, Italy and Turkey) (Buonfrate, 2014). Mycetoma involving the foot is called "Madura foot" and it accounts for 68.7% of mycetoma cases. Fungi are involved as causative agents of mycetoma in 41.7% of cases (Eumycetoma) and *Madurella mycetomatis* is the most common causative agent, accounting for 24.3% of the cases.

The recommended treatment is itraconazole 200-400 mg/day for 6-9 months, in combination with surgery if necessary (van de Sande, 2013, Zijlstra 2016, van Belkum 2013).

Key words:
Madura foot, Actinomadura madurae, Sequencing 16S r-DNA, Ribosomal RNA.

Corresponding author:
Filippo Bartalesi
E mail: bartalesif@aou-careggi.toscana.it

Bacteria are causative agents of mycetoma in 50.8% of the cases, with *Actinomadura madurae* being the most common species isolated (20.1%) (van de Sande, 2013). The radiological findings of Madura foot are not specific and computed tomography scans (CT) and magnetic resonance imaging (MRI) point out the soft tissue and joint and bone involvement, allowing a classification of the severity of infection (El Shamy, 2012; Ispoglou, 2013). The differential diagnosis includes infectious (tuberculosis, fungal or bacterial infections) and non-infectious skin diseases (bone or soft tissue cancer).

The isolation of the causative agent requires prolonged bacterial cultures in aerobic and anaerobic conditions (until 10 days at 35°-37°C). Recommended media are Columbia agar, Brain Heart Infusion, Lowenstein-Jensen and Sabouraud glucose agar (Nenoff, 2015).

Molecular detection of the pathogen directly on biopsy specimens by PCR and sequencing may be a rapid diagnostic tool for this rare disease and may allow identification of the causative agent in case of negative cultures (Salipante, 2013). Moreover, the use of universal fungal internal transcribed spacer (ITS) primers for amplification and sequencing could help the identification of cases of Madura foot caused by fungi (Desnos-Ollivier, 2006).

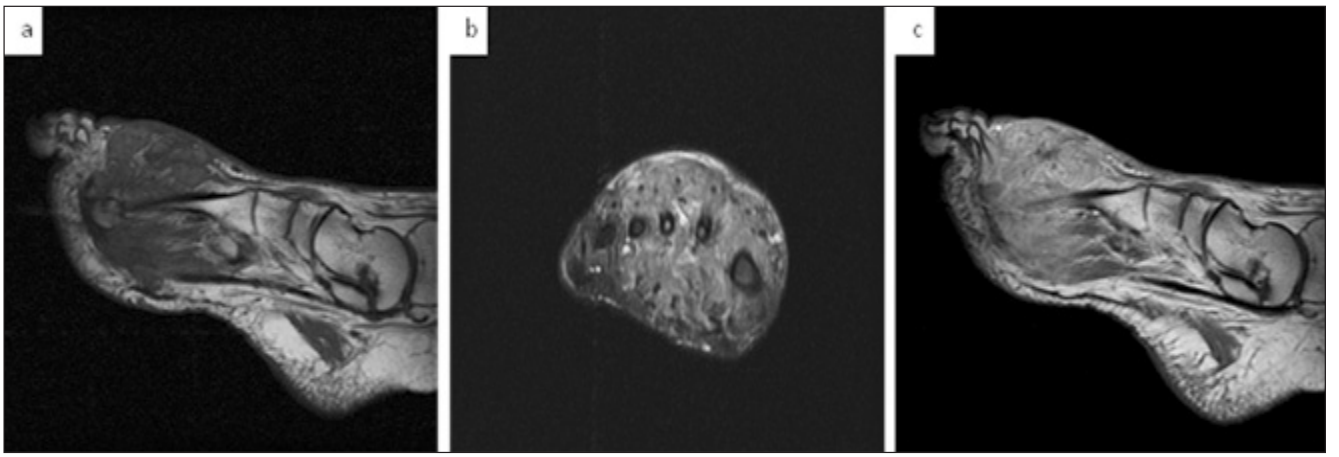

Figure 1 - *MR image of the Madura foot. a soft tissue ill-defined mass involved the dorsal aspect of the forefoot and the subcutaneous tissue, infiltrating intermetatarsal spaces and the underlying plantar muscles. No microabscesses or bone involvement were observed.*
a) sagittal T1-weighted image: conglomerate areas of multiple, discrete, small round nodules (3-5 mm) isointense compared with muscle; b) coronal STIR (short tau inversion recovery) image: hyperintense conglomerate areas of nodules which were separated by a low signal intensity rim. In the centre of some of these nodules there is a tiny hypointense focus, resulting in the characteristic dot-in-circle sign, which indicates mycetoma grains; c) sagittal T1-weighted image after intravenous gadolinium contrast administration: the granulomatous tissue shows diffuse hyperintensity of signal with non-enhanced central foci corresponding to mycetoma grains.

CASE REPORT

We describe a case of Madura foot in a 48 year-old Italian woman, working as a florist. She did not refer travel or trauma. In March 2012, she presented a swelling of the dorsum of the right foot. In October 2013 an MRI scan showed an infiltrative mass between II-III and III-IV metatarsus, extending to flexor tendons, without bone involvement (*Figure 1*). The soft tissue biopsy showed an inflammatory reaction, but the orthopaedics who assessed the patient did not prescribe any therapy or further diagnostic procedures. In February 2014, a second biopsy confirmed an inflammatory necrotic exudative reaction and the culture was positive for methicillin-resistant *Staphylococcus epidermidis*. At that time *S. epidermidis* was considered the potential causative agent of the infection and daptomycin 8 mg/kg/day was given for 45 days in combination with rifampin 600 mg/day for 10 days, with partial improvement. After a few weeks the patient again presented pain and worsening of the swelling in the dorsum of the right foot. A new MRI did not show significant changes. In July 2015, the patient was referred to the Infectious

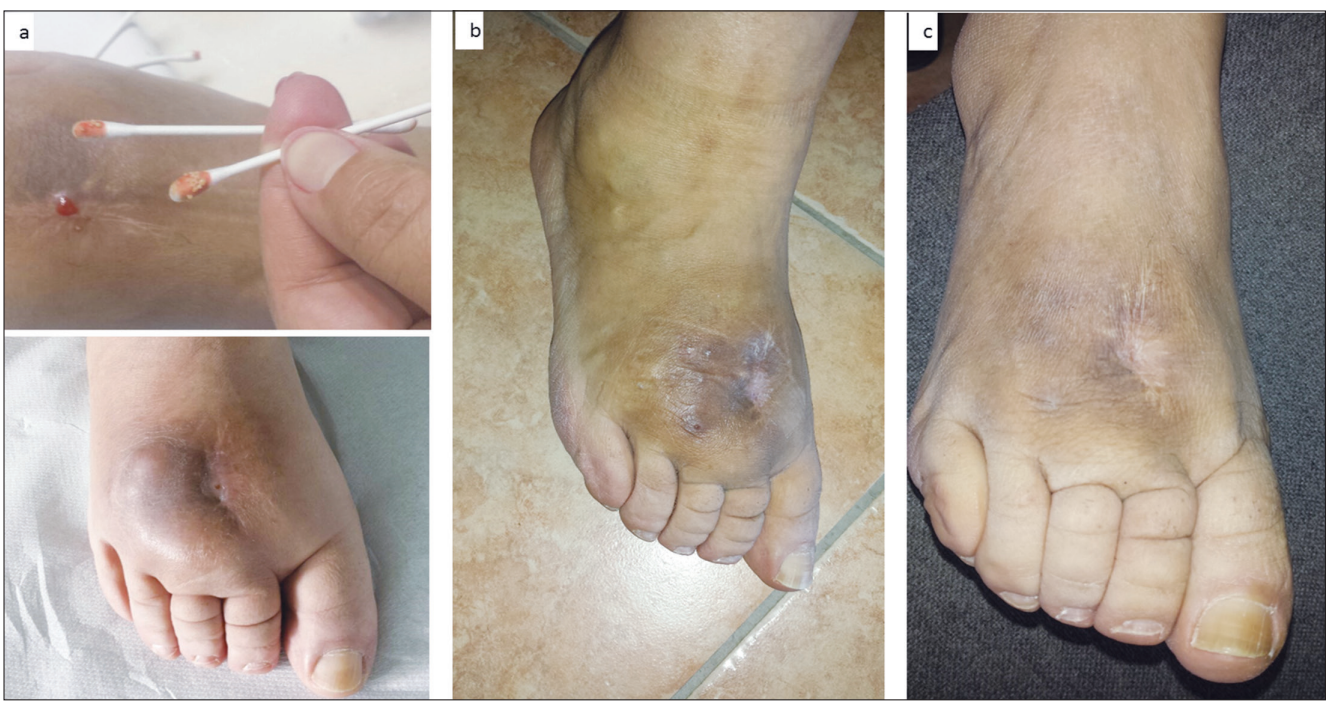

Figure 2 - *Madura foot due to Actinomadura madurae.*
a) at the start of the therapy a fistula presented on the dorsum of the right foot with grains in the purulent drainage; b) After 3 months of therapy; c) After 6 months of therapy.

and Tropical Diseases Unit of Careggi Hospital, where she underwent a new biopsy for the suspected diagnosis of Madura foot. All aerobic and anaerobic cultures, incubated for 10 days, performed on Columbia agar 5%, Chocolate agar PolyViteX plates (bioMérieux, France), Shaedler KKV Agar (Liophilchem, Italy) Sabouraud Dextrose Agar, Sabouraud Dextrose Broth, Thioglycollate Fluid Medium and Nutrient Broth (MEUS, Italy), resulted negative. Si-

Table 1 - *European cases of Actinomycetoma.*

Author, year, country	*Specialist making diagnosis*	*Country of birth of the patient*	*Risk factors*	*Time from onset to diagnosis*	*Bone involvement*	*Culture (media)*	*PCR*	*Etiology*	*Surgery*	*Therapy (duration, mm)*
Balabanoff, 1980, Bulgary	Dermatologist	Bulgary	No	11 years	Yes	Positive (NR)	No	*A. madurae*	Refused	Nitroxoline (NR)
Balabanoff, 1980, Bulgary	Dermatologist	Bulgary	NR	4 years	NR	Positive (NR)	No	*N.asteroides*	NR	NR
Balabanoff, 1980, Bulgary	Dermatologist	Bulgary	NR	4 years	NR	Positive (NR)	No	*Nocardia spp*	NR	NR
Balabanoff, 1980, Bulgary	Dermatologist	Bulgary	NR	7 years	NR	Positive (NR)	No	*Nocardia spp*	NR	NR
Balabanoff, 1980, Bulgary	Dermatologist	Bulgary	NR	2 years	NR	Positive (NR)	No	*A. madurae*	NR	NR
Binazzi, 1982, Italy	Dermatologist	Italy	No	5 years	Yes	Positive (NR)	No	*A. madurae*	No	Penicillin, cam, dox, SXT, rifampin, lincomycin (NR); ketoconazole (NR)
Pelzer, 2000, Germany	Dermatologist	Greece	Trauma	1 year	No	Positive (SGA, rice agar, Kimmig's agar)	No	*N.asteroides, Sporothrix schenckii*	No	SXT + itraconazole (7)
Rigopoulos, 2000, Greece	Dermatologist	Albania	Trauma	4 years	Yes	NR	No	*A. madurae*	No	Penicillin + SXT (25 days) A+SXT (1) Daps+mino (lost follow up)
Papaioannide, 2001, Greece	Internal Medicine specialist	Albania	Farmworker	4 years	No	NR	No	*Actinomadura spp*	No	NR
Ispoglou, 2003, Greece	Internal Medicine specialist	Greece	No	5 years	Yes	NR	No	*A. madurae*	No	Daps+S (18)
Usai, 2005, Italy	Dermatologist	Italy	Farmworker	NR	No	Positive (BA, CA)	NR	*A. madurae*	No	I + Dox (NR); Relapse: LNZ (NR)
De Palma, 2006, Italy	Orthopaedic surgeon	Albania	No	5 years	Yes	Positive (SGA, CBA)	No	*A. madurae*	No	SXT + A (2) then SXT (6)
Gunduz, 2006, Turkey	Dermatologist	Turkey	Nail trauma	15 years	No	Positive (NR)	No	*NR*	No	Ciprofloxacin + itraconazole + SXT (14)
Buonfrate, 2014, Italy	Infectious diseases specialist (referred by orthopaedic)	Albania	Forest ranger	12 years	NR	Positive (NR)	NR	*A. madurae*	No	NR (24)
Buonfrate, 2014, Italy	Infectious diseases specialist	Albania	Woodcutter	19 years	NR	Positive (NR)	NR	*A. madurae*	Yes	NR (24)
Case presented in this article	Infectious diseases specialist (referred by orthopaedic)	Italy	Florist?	3 years	No	Negative (CBA, SGA, CA)	16S	*A. madurae*	No	Rifampin + A + SXT

A: amikacin; BA: blood agar; CA: chocolate agar; Cam: chloramphenicol; CBA: Columbia blood agar ; Daps: dapsone; Dox: doxicycline; I: imipenem; LNZ: linezolid; Mino: minocycline; NR: not reported; PCR: polymerase chain reaction; S: streptomycin; SGA: Sabouraud glucose agar; SXT: co-trimoxazole.

multaneously, total DNA was extracted from the biopsy with a Nuclisens® EasyMAg® instrument (bioMeriéux) and amplified with universal 16S rDNA primers D88 and E94 (Paster, 2001). The PCR tested positive, and the amplicon sequence, determined on both strands, yielded a 99.1% identity with *A. madurae* DSM 43067 (accession no. NR_026343). After the last biopsy a fistula presented on the dorsum of the right foot with grains in the purulent drainage. The patient started therapy with rifampin 600 mg/day, trimethoprim-sulfamethoxazole (SXT) 960 mg three times a day, and amikacin 1 g for the first 15 days. After 3 months of treatment the pain had disappeared and the swelling subsided. The patient was reassessed also after about 6 months (February 2016) and the lesion had fully healed (*Figure 2*).

We reviewed the literature on Madura foot using the electronic database Pubmed and inserting these search terms: Madura foot, Europe, European countries, Actinomycetoma. We restricted the review to bacterial causative agents, excluding localizations other than foot or fungal etiology. We found 16 bacterial Madura foot reports. Including the present report in the analysis, 11 of them were caused by *Actinomadura* spp (64.7%), 5 cases (31.2%) presented bone involvement (*Table 1*) and in one of those a below-knee amputation was performed. Six of the 17 patients were migrants from other European countries (mostly from Albania). The reviewed cases showed an extremely high median time from onset to diagnosis of 6.7 years (range 1-19 years). The difficulty of an early diagnosis is probably due to several factors, namely the rarity of illness and the difficulties of the microbiological diagnosis.

Moreover, the radiological diagnosis of Madura foot is challenging, and very often misdiagnosed as an infiltrative lesion. A multidisciplinary approach is needed, considering also that in addition to the present case only 2 other cases were referred to infectious diseases specialist, while the other patients were assessed by dermatologists (58.8%), internal medicine specialists (11.8%) and orthopaedics (5.9%). Probably the implementation of a surveillance system, the involvement of an infectious diseases specialist, with experience in tropical diseases, and the availability of a microbiology unit performing feasible and rapid molecular diagnostic tests could result in an earlier diagnosis and an optimal antibiotic therapy. In our case the molecular detection of the pathogen directly on biopsy specimens by PCR and sequencing allowed us to identify the causative agent and to prescribe the appropriate antibiotic therapy.

As for treatment, different antimicrobial drugs were used in the reported cases due to *A. madurae*, with a wide range of length of therapy (8- 24 months), evidencing the lack of clinical studies to determine the best therapeutic approach. The most common treatment was an association of two or more antimicrobial agents, including penicillin, aminoglycosides, dapsone, SXT, oxazolidinones, and quinolones for several months (up to 24 months) (Bettesworth, 2009; Welsh, 2012). *In vitro A. madurae* is sensitive to amikacin, SXT, linezolid and quinolones (Vera-Cabrera, 2004). However, Clinical and Laboratory Standard Institute breakpoints are validated for *Nocardia* spp and can only tentatively be used for actinomycetes (Woods, 2011). Due to the growing number of migrants to European countries and of travelers to tropical countries, an increase in autochthonous and imported cases of Madura foot in Europe is to be expected.

The present report points out the importance of the knowledge of this rare but difficult-to-treat and, above all, difficult-to-diagnose infection.

References

Balabanoff V.A. (1980). Mycetomas originated from South-East Bulgaria (author's transl). *Ann Parasitol Hum Comp.* **55**, 605-613.

Bettesworth J., Gill K., Shah J. (2009). Primary actinomycosis of the foot: a case report and literature review. *J Am Col Certif Wound Spec.* **1**, 95-100.

Binazzi M., Papini M. (1983). Madura foot by *Actinomadura madurae*. A further report on an autochthonous Italian case. *Mykosen.* **26**, 298-304.

Buonfrate D., Gobbi F., Angheben A., Marocco S., Farina C., Van De Ende J., Bisoffi Z. (2014). Autochthonous cases of mycetoma in Europe: report of two cases and review of literature. *PLoS One.* **9**, e100590.

De Palma L., Marinelli M., Pavan M., Manso E., Ranaldi R. (2006). A rare European case of Madura Foot due to actinomycetes. *Joint Bone Spine.* **73**, 321-324.

Desnos-Ollivier M., Bretagne S., Dromer F., Lortholary O., Dannaoui E. (2006). Molecular identification of black-grain mycetoma agents. *J Clin Microbiol.* **44**, 3517-3523.

El Shamy M.E., Fahal A.H., Shakir M.Y., Homeida M.M. (2012). New MRI grading system for the diagnosis and management of mycetoma. *Trans R Soc Trop Med Hyg.* **106**, 738-742.

Gunduz K., Orguc S., Demireli P., Inanir I., Surucuoglu S., Ovali G.Y. (2006). A case of mycetoma successfully treated with itraconazole and co-trimoxazole. *Mycoses.* 49, 436-438.

Ispoglou S.S., Zormpala A., Androulaki A., Sipsas N.V. (2013). Madura foot due to *Actinomadura madurae*: imaging appearance. *Clin Imaging.* **27**, 233-235.

Nenoff P., van de Sande W.W., Fahal A.H., Reinel D., Schofer H. (2015). Eumycetoma and actinomycetoma - an update on causative agents, epidemiology, pathogenesis, diagnostics and therapy. J Eur Acad Dermatol Venereol. doi: 10.1111/jdv.13008.

Papaioannides D., Akritidis N.K. (2001). Painless foot swelling with a chronic purulent discharge. *West J Med.* **174**, 96-97.

Paster B.J., Boches S.K., Galvin J.L., Ericson R.E., Lau C.N., Levanos V.A., et al. (2001). Bacterial diversity in human subgingival plaque. *J Bacteriol.* **183**, 3770-3783.

Pelzer K., Tietz H.J., Sterry W., Haas N. (2000). Isolation of both *Sporothrix schenckii* and *Nocardia asteroides* from a mycetoma of the forefoot. Br J Dermatol. **143**, 1311-1315.

Rigopoulos D., Mavridou M., Nicolaidou E., Christofidou E., Antoniou C., Stratigos A., Katsambas A. (2000). Mycetoma due to actinomycetes: a rare entity in Europe. *Int J Dermatol.* **39**, 557-558.

Salipante S.J., Sengupta D.J., Hoogestraat D.R., Cummings L.A., Bryant B.H., Natividad C., et al. (2013). Molecular diagnosis of *Actinomadura madurae* infection by 16S rRNA deep sequencing. *J Clin Microbiol.* **51**, 4262-4265.

Usai F., Carluccio A., Caddeo R., Pastorelli C., Saddi B. (2005). Madura foot: descrizione di un caso clinico. *Microbiologia Medica.* **20**.

van Belkum A., Fahal A.H., van de Sande W.W.J. (2013). Mycetoma caused by *Madurella mycetomatis*: a completely neglected medico-social dilemma. *Adv Exp Med Biol.* **764**, 179-189.

van de Sande W.W. (2013). Global burden of human mycetoma: a systematic review and meta-analysis. *PLoS Negl Trop Dis.* **7**, e2550.

Vera-Cabrera L., Ochoa-Felix E.Y., Gonzalez G., Tijerina R., Choi S.H., Welsh O. (2004). *In vitro* activities of new quinolones and oxazolidinones against *Actinomadura madurae*. *Antimicrob Agents Chemother.* **48**, 1037-1039.

Welsh O., Vera-Cabrera L., Welsh E., Salinas M.C. (2012). Actinomycetoma and advances in its treatment. Clin Dermatol 30, 372-81.

Woods G.L., Brown-Elliott B.A., Conville P.S., Desmond P.E., Hall G.S., Lin G., et al. (2011). Susceptibility testing of mycobacteria, nocardia and other aerobic actinomycetes; approved standard CLSI document M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.

Zijlstra E.E., van de Sande W.W.J., Welsh O., Mahgoub E.S., Goodfellow M., Fahal A. (2016). Mycetoma: a unique neglected tropical disease. *Lancet Infect Dis.* **16**, 100-112.