Review of 21 cases of mycetoma from 1991 to 2014 in Rio de Janeiro, Brazil

Felipe Maurício Soeiro Sampaio*, Bodo Wanke, Dayvison Francis Saraiva Freitas, Janice Mery Chicharino de Oliveira Coelho, Maria Clara Gutierrez Galhardo, Marcelo Rosandiski Lyra, Maria Cristina da Silva Lourenço, Rodrigo de Almeida Paes, Antonio Carlos Francesconi do Valle

National Institute of Infectious Diseases, Oswaldo Cruz Foundation - Rio de Janeiro - Brazil

* felipemauricio@uol.com.br

Abstract

Mycetoma is caused by the subcutaneous inoculation of filamentous fungi or aerobic filamentous bacteria that form grains in the tissue. The purpose of this study is to describe the epidemiologic, clinical, laboratory, and therapeutic characteristics of patients with mycetoma at the Oswaldo Cruz Foundation in Rio de Janeiro, Brazil, between 1991 and 2014. Twenty-one cases of mycetoma were included in the study. There was a predominance of male patients (1.3:1) and the average patient age was 46 years. The majority of the cases were from the Southeast region of Brazil and the feet were the most affected anatomical region (80.95%). Eumycetoma prevailed over actinomycetoma (61.9% and 38.1% respectively). Eumycetoma patients had positive cultures in 8 of 13 cases, with isolation of Scedosporium apiospermum species complex (n = 3), Madurella mycetomatis (n = 2) and Acremonium spp. (n = 1). Two cases presented sterile mycelium and five were negative. Six of 8 actinomycetoma cases had cultures that were identified as Nocardia spp. (n = 3), Nocardia brasiliensis (n = 2), and Nocardia asteroides (n = 1). Imaging tests were performed on all but one patients, and bone destruction was identified in 9 cases (42.68%). All eumycetoma cases were treated with itraconazole monotherapy or combined with fluconazole, terbinafine, or amphotericin B. Actinomycetoma cases were treated with sulfamethoxazole plus trimethoprim or combined with cycles of amikacin sulphate. Surgical procedures were performed in 9 (69.2%) eumycetoma and in 3 (37.5%) actinomycetoma cases, with one amputation case in each group. Clinical cure occurred in 11 cases (7 for eumycetoma and 4 for actinomycetoma), and recurrence was documented in 4 of 21 cases. No deaths were recorded during the study. Despite of the scarcity of mycetoma in our institution the cases presented reflect the wide clinical spectrum and difficulties to take care of this neglected disease.

Author summary

Mycetoma is a major health problem in tropical areas and is prevalent among people of low socio-economic status. As in many other regions of the world, the incidence and prevalence of mycetoma in Brazil is unknown. This study describes some aspects of mycetoma...
patients in 24 years of experience at the National Institute of Infectious Diseases at the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil and contribute to the knowledge on mycetoma epidemiology globally.

Introduction

Mycetoma is a chronic subcutaneous infections caused by the inoculation of filamentous fungi (eumycetoma) or aerobic filamentous bacteria (actinomyctoma) that form grains in the affected tissues [1]. It’s considered a neglected disease by the World Health Organization (WHO) since 2016 and remains without any control program for prevention or surveillance [1, 2].

Mycetoma occurs worldwide and prevails in tropical and subtropical regions, especially in sub-Saharan areas of Africa, India, and Mexico [3,4]. In South America, cases have been reported in Venezuela, Colombia, Brazil, and Argentina [1,3,5]. The incidence and prevalence of mycetoma in Brazil are unknown, since it is not considered a public health problem, as its frequency is smaller than other diseases such as sporotrichosis, tuberculosis, leprosy, and dengue (the latter two are classified as neglected diseases by the WHO) [6]. Mycetoma evolves slowly in its clinical manifestation. Laboratory diagnosis and treatment are difficult, presenting significant medical, occupational and socioeconomic impacts [2,7].

In this study, we describe the epidemiological, clinical, laboratory, and therapeutic aspects of patients treated at a reference hospital in Rio de Janeiro, Brazil, between 1991 and 2014.

Methods

Ethics

The study was approved by the Research Ethics Committee of the INI / Fiocruz, on November 25, 2013 under the number 477.037. All participants gave their written consent, with the exception of those who died before the study. In all cases the identity and information of each patient were preserved.

Laboratorial diagnosis

Histological examination was performed using haematoxylin-eosin, Grocott’s methenamine silver, Periodic acid–Schiff, and Gram-Brown-Brenn stains. Biopsy specimens were submitted for direct microscopic examination with 10% potassium hydroxide where grains were classified according to their size, shape, colour, consistency and presence of hyphae or filamentous bacteria. Culture on Sabouraud’s Dextrose Agar 2% and Mycobiotic Agar was performed for eumycotic grains and in/on Lowenstein-Jensen medium, defibrinated sheep blood agar chocolate agar and thioglycolate medium with resazurina for actinomycotic grains.

Bacterial and fungal etiologic agents were identified by examination of the colonies in culture.

Imaging tests

Ultrasound, radiography, computerized tomography (CT), and magnetic resonance imaging (MRI) were performed to determine deep tissue and bone involvement and presence of grains.
Treatment

Actinomycetoma patients were treated with oral sulfamethoxazole-trimethoprim (SMX-TMP) 800/160 mg BD, alone or in combination with alternate cycles of 15 mg/kg/day intravenous amikacin for three weeks in cases with bone destruction. Other antimicrobials were given in case of secondary infections. Eumycetoma patients were treated with itraconazole (200 mg, BD) alone or, in cases without consistent clinical response after six months, in combination with fluconazole 200 mg/day, terbinafine 250 mg/day, or amphotericin B 1 mg/kg. Surgical treatment was indicated for small and delimited lesions and in cases of bone destruction. Amputation was indicated in cases lacking a satisfactory antimicrobial response associated to severe bone destruction of the affected segment.

Follow-up

The patients were followed-up bimonthly at the outpatient clinic to assess clinical responses to treatment and drug side effects. A complete cure was defined with the healing of lesions, bone remodelling, and absence of grains upon imaging examination. After the determination of the clinical cure, outpatient follow-up turned annual, to assess the possibility of recurrence.

Statistics

Data retrieved from patients records were analysed using descriptive statistics with the Statistical Package for the Social Sciences, version 20.0. Data were summarized as percentages for categorical variables and mean, median, and range for continuous variables.

Results

A total of 21 mycetoma cases were included in the present study: 13 eumycetoma and 8 actinomycetoma patients.

The main sociodemographic aspects of the mycetoma patients are summarised in Table 1. In brief, the male to female ratio was 1.3:1, and the mean age was 46 years old (range 28–93 years). However, the mean age for eumycetoma was 51.3 years old and 38.6 years old for actinomycetoma. The non-white ethnicity/race predominated with 66.66%. Most patients (71.43%) came from the southeast region of Brazil, and 28.57% came from the northeast region. These regions correspond to the possible original infection sites.

Comorbidities occurred in 10 patients. Eight of them presented a single comorbidity and the others had two comorbidities. In general, high blood pressure, diabetes mellitus, HIV positive (Figs 1 and 2), and asthma were found.

The time from onset of signs and symptoms to medical care ranged from 2 to 420 months (mean = 77.68 months). The average time was higher for eumycetoma (mean = 105.76 months) than actinomycetoma (mean = 36.75 months).

The foot (Figs 3, 4 and 5) was affected in 17 cases (80.9%), the thigh was affected in two cases, and the hand and ankle were affected in one case each. A history of trauma was reported in 17 (80.9%) cases.

The grains were mainly identified through histopathological examination with 90.4% positivity in these methods and 9.6% through direct microscopy. We retrieved the etiological agents in 61.5% eumycetoma cases (Table 2) and in 75% of actinomycetoma cases (Table 3).

In the eumycetoma group, the *Scedosporium apiospermum* species complex was identified in three cases, *Madurella mycetomatis* was isolated from two cases and *Acremonium* sp. was isolated from one case. From the remaining two patients, the isolated filamentous fungi could
not be identified, as they only produced hyphae without any conidia or spores, and therefore they were named Mycelia sterilia (cases 7 and 8, Table 2). It is important to note that these two organisms were consistently isolated as pure cultures in at least three consecutive mycological examinations.

In the actinomycetoma group we isolated *Nocardia* spp. from three cases, *Nocardia brasiliensis* from two cases and *Nocardia asteroides* from one case (Table 3). In two cases the culture were negative.

All patients underwent radiography of the affected site with exception of patient 7 of Table 3, who underwent complete excision with security margin of the lesion during the diagnostic procedure (Fig 6).
Ultrasonography was performed in 18 cases, with the observation of subcutaneous nodules in all of them. Ten patients underwent CT scans and seven patients underwent MRI. Bone involvement was present in 9 cases, five from eumycetoma and four from actinomycetoma. Secondary bacterial infection was diagnosed in four cases, two of them had *Staphylococcus aureus* associated infection treated with systemic antibiotics guided by susceptibility tests. The other two cases were treated empirically.

Patients with eumycetoma received 200 mg BD itraconazole alone (8/13) or in combination with 200 mg/day fluconazole (3/13), or 250 mg/day terbinafine (2/13). In case 1 (Table 2), when the patient became pregnant during itraconazole treatment, this drug was suspended and we tried to use liposomal amphotericin B due to clinical worsening, without success.

Actinomycetoma patients received 800/160 mg sulfamethoxazole-trimethoprim BD in most of cases (75%). As monotherapy in five cases, one case with cycles of 15 mg/kg/day amikacin sulphate and another case received 500 mg cephalexin four times a day. The used of cephalexin occurred because of the repeated secondary bacterial infection. The case 7 (Table 3) with a small and well delimited lesion in lower limb underwent complete excision with security margin and therefore was not treated with antimicrobials. The case 8 (Table 3), who presented with multiple foci of bone destruction, was submitted to amikacin cycles, which had to be stopped after the fifth cycle due to changes in audiometry and increased creatinine levels, without lifelong clinical consequences. Itraconazole was used in all cases and combined with another antifungal agents (38%) in refractory cases.

The average treatment time was 35.04 months (range 6–144 months). The mean treatment time was 42.53 months for eumycetoma and 22.87 months for actinomycetoma. The average
Fig 2. HIV patient with actinomycetoma after treatment.
doi:10.1371/journal.pntd.0005301.g002

Fig 3. Foot affected with actinomycetoma.
doi:10.1371/journal.pntd.0005301.g003
Fig 4. Right foot affected with actinomycetoma and the left foot without disease.

doi:10.1371/journal.pntd.0005301.g004

Fig 5. Right foot affected with actinomycetoma after treatment.

doi:10.1371/journal.pntd.0005301.g005
treatment time for patients with bone destruction was 70 months (median 55 months) for eumycetoma cases and 33 months (median 36 months) for actinomycetoma cases.

Amputation was recommended for three patients with eumycetoma, one of them accepted the procedure and the other two remain receiving drug treatment until now. In the actinomycetoma group, one patient accepted amputation. Surgical excision of small lesions were performed in nine eumycetoma patients and three actinomycetoma patients.

Table 2. Main characteristics of 13 patients with Eumycetoma.

| Patient | Sex | Etiologic agent          | Grains | Treatment      | Time for treatment (months) | Surgery | Bone involvement | Outcome |
|---------|-----|--------------------------|--------|----------------|-----------------------------|---------|------------------|---------|
| 1       | F   | Madurella mycetomatis    | H      | ITZ / AMB      | 24                          | Yes*    | Yes              | Cure    |
| 2       | M   | Madurella mycetomatis    | H/DM   | ITZ + FLZ      | 60                          | Yes     | No               | Cure    |
| 3       | M   | Acremonium sp.           | H      | ITZ + FLZ      | 144                         | Yes     | Yes              | No Cure |
| 4       | M   | Scedosporium apiospernum| H/DM   | ITZ            | 46                          | Yes     | No               | Cure    |
| 5       | M   | Scedosporium apiospernum| H/DM   | ITZ + TBF      | **                          | No      | No               | **      |
| 6       | M   | Scedosporium apiospernum| H      | ITZ + TBF / ITZ| 74                          | No      | Yes              | ***     |
| 7       | M   | Filamentous fungi        | H      | ITZ            | 123                         | Yes     | No               | Cure    |
| 8       | F   | Filamentous fungi        | H/DM   | ITZ            | **                          | No      | Yes              | **      |
| 9       | F   | Negative culture         | H/DM   | ITZ            | 24                          | Yes     | No               | Cure    |
| 10      | F   | Negative culture         | H      | ITZ            | 36                          | Yes     | Yes              | No cure |
| 11      | M   | Negative culture         | H      | ITZ            | 7                           | Yes     | No               | Cure    |
| 12      | F   | Negative culture         | H      | ITZ            | 9                           | Yes     | No               | Cure    |
| 13      | M   | Negative culture         | H      | ITZ            | 6                           | No      | No               | ***     |

F: female; M: male
H: histopathology; DM: direct microscopy; ITZ: itraconazole; FLZ: fluconazole; TBF: terbinafine; AMB: amphotericin B.
*Amputation;
**Abandon of follow up;
***Still in treatment.

doi:10.1371/journal.pntd.0005301.t002

treatment time for patients with bone destruction was 70 months (median 55 months) for eumycetoma cases and 33 months (median 36 months) for actinomycetoma cases.

Amputation was recommended for three patients with eumycetoma, one of them accepted the procedure and the other two remain receiving drug treatment until now. In the actinomycetoma group, one patient accepted amputation. Surgical excision of small lesions were performed in nine eumycetoma patients and three actinomycetoma patients.

Table 3. Main characteristics of 8 patients with Actinomycetoma.

| Patient | Sex | Etiologic agent          | Grains | Treatment      | Time for treatment (months) | Surgery | Bone involvement | Outcome |
|---------|-----|--------------------------|--------|----------------|-----------------------------|---------|------------------|---------|
| 1       | M   | Nocardia brasiliensis    | H      | TMP + SMX      | 48                          | Yes     | Yes              | ***     |
| 2       | M   | Nocardia brasiliensis    | H/DM   | TMP + SMX      | 38                          | No      | Yes              | **      |
| 3       | F   | Nocardia sp.             | DM     | TMP + SMX      | 36                          | No      | No               | Cure    |
| 4       | M   | Nocardia sp.             | DM     | TMP + SMX      | 10                          | No      | No               | Cure    |
| 5       | M   | Nocardia sp.             | H      | TMP + SMX      | 8                           | No      | No               | ***     |
| 6       | F   | Nocardia asteroides      | H      | TMP + SMX / cefalexin | 19 | Yes * | Yes | Cure |
| 7       | F   | Negative culture         | H      | —              | 0                           | Yes     | No               | Cure    |
| 8       | F   | Negative culture         | H      | TMP + SMX / 5 cycles of amikacine | 24 | No | Yes | *** |

F: female; M: male
H: histopathology; DM: direct microscopy; TMP + SMX: Trimethoprim/sulfamethoxazole.
* Amputation;
** Abandon of follow up;
*** Still in treatment.

doi:10.1371/journal.pntd.0005301.t003
Fig 6. Radiography showing bone destruction.
doi:10.1371/journal.pntd.0005301.g006
Clinical cure occurred in 11 (52.38%) of all cases. Of the 13 eumycetoma patients, seven were cured, two abandoned follow up and another two patients are still under antifungal treatment. Of the five eumycetoma patients with bone involvement, one underwent amputation, two remained in treatment, one remain under observation and one abandoned treatment.

Of the eight actinomycetoma patients, four were cured, one abandoned the treatment and three are under treatment. Of the four actinomycetoma cases with bone involvement, one patient was underwent amputation, two remained in treatment and one abandoned treatment.

If we consider the cure without sequelae (amputation), the rate falls to 42.8%.

Recurrence of infection was observed in four patients: one with actinomycetoma and three with eumycetoma. The time to recurrence was 24 months for the actinomycetoma case and ranged from 8 to 96 months (mean = 36.6 months) for eumycetoma cases.

Treatment dropouts was high (23%) and recurrence was also frequent (19%) and prevailed in patients that had undergone surgery, especially in the eumycetoma group.

The broad range of treatment duration until clinical cure (6–114 months) was a striking observation of this study.

Discussion and review of the literature

The 21 mycetoma cases diagnosed in the 24-year period of this study demonstrate the low frequency of mycetoma in our institution at Rio de Janeiro, Brazil. Most reports of mycetoma in Brazil describe one or a few cases, reinforcing the scarcity of the disease in this country. To achieve a better comprehension on this subject we performed a search of articles on PubMed (from 1980 to 2014) using the MESHterms “Mycetoma”, “Actinomycetoma”, and “Eumycetoma” alone or in combination with “Brazil”. During this period, 272 mycetoma cases were reported (Table 4). This number is smaller than that observed in Sudan and Mexico [8, 9, 10]. For instance, in Mexico, where 483 mycetoma cases were diagnosed at a single hospital during the same period [11]. In 2013, van de Sande et al. [1] estimated the prevalence of mycetoma cases in Mexico and the Sudan as 0.15 and 1.81 cases per 100,000 inhabitants, respectively, compared to the prevalence of less than 0.001 per 100,000 inhabitants in Brazil.

The predominance of eumycetoma in our study might not represent the real scenery of mycetoma in Brazil, as the Brazilian literature reveals a higher frequency of actinomycetoma (Table 4) [12, 13, 14, 15]. The involvement of male individuals above 30 years old with an acral location likely due to increased risk exposure during labour activity without safety equipment is in accordance with mycetoma characteristics [5, 16, 17].

Although eumycetoma and actinomycetoma share similar clinical aspects, we noted that eumycetoma cases usually tend to be more silent and chronic, while actinomycetoma cases were more inflammatory and painful. This fact may explain why patients with eumycetoma take longer to seek medical care.

We noted that six of our patients moved from the Northeast region of Brazil to the Rio de Janeiro state, in the Southeast region, probably attracted for job possibilities in a state with higher socio-economic index, higher urbanization of population and better health infrastructure. For this reason, we assume that, for these patients, the place where infection occurred was not in Rio de Janeiro.

Comorbidities are not associated to more severe or atypical forms of mycetoma and there are no changes in the course of mycetoma in the HIV infected patient [18, 19, 20]. Although it requires further investigation, pregnancy may be linked to more severe clinical course of mycetoma [21, 22, 23, 24] as in case 1 (Table 2) that developed severe bone destruction during pregnancy, resulting in amputation of the affected limb [25].
| Reference | Year | Study | Classification | Number of cases | Etiologic agent | Region |
|-----------|------|-------|----------------|----------------|----------------|--------|
| [73]      | 1980 | Clinical report + review | unknown | 4 | Unknown | Amazonas (North) |
| [49]*     | 1980 | Clinical report | Eumycetoma | 1 | Petriellidium boydii (Scedosporium apiospermum) | - |
| [50]      | 1981 | Clinical report | Eumycetoma | 1 | Petriellidium boydii (Scedosporium apiospermum) | Minas Gerais (Southeast) |
| [12]      | 1981 | Retrospective (1944–1978) | Eumycetoma | 41 113 | Unknown (Histopathological aspects) | Sao Paulo (South) |
|           |      |       | Eumycetoma |  |   |                     |
| [27]      | 1982 | Clinical report | Actinomycetoma | 6 | Nocardia sp. | Sao Paulo (Southeast) |
| [39]*     | 1982 | Clinical report | Eumycetoma | 1 | Madurella grisea | - |
| [38]      | 1984 | Clinical report + review | Actinomycetoma | 1 | Actinomadura madurae | Sao Paulo (Southeast) |
| [74]      | 1986 | Case series | Actinomycetoma | 4 | Nocardia brasiliensis | Rio Grande do Sul (South) |
| [75]      | 1988 | Retrospective | Unknown | 2 | Unknown | Amazonas (North) |
| [46]      | 1988 | Clinical report + review | Eumycetoma | 1 | Acremonium falciforme | Bahia (Northeast) |
| [52]*     | 1988 | Clinical report | Eumycetoma | 1 | Exophiala jeanselmei | - |
| [40]*     | 1989 | Clinical report | Eumycetoma | 1 | Madurella grisea | - |
| [41]      | 1989 | Clinical report | Eumycetoma | 1 | Madurella grisea | - |
| [76]      | 1990 | Comunication | Actinomycetoma | 1 | Unknown | Ceará (Northeast) |
| [42]      | 1991 | Clinical report | Eumycetoma | 1 | Madurella grisea | Goiás (Midwest) |
| [77]*     | 1991 | - | - | - | - | Goiânia-Goiás (Midwest) |
| [13]      | 1992 | Clinical report + review | Actinomycetoma | 2 | Actinomadura madurae | Rio de Janeiro (Southeast) |
|           |      |       |       |   | **** Review: Actinomycetoma 61 cases Eumycetoma 33 cases | Pernambuco (Northeast) |
| [43]      | 1992 | Clinical report | Eumycetoma | 2 | Madurella grisea | Bahia (Northeast) |
| [33]      | 1993 | Clinical report | Actinomycetoma | 1 | Nocardia asteroides | Rio de Janeiro (Southeast) |
| [14]      | 1993 | Retrospective 1978–1989 | Eumycetoma | 13 28 | Madurella grisea—3 Scedosporium apiospermum—2 Madurella mycetomatis—1 Culture negative—7 Nocardia brasiliensis—13 Actinomadura madurae—1 Actinomadura pelletieri—1 Nocardia asteroides—1 Culture negative—12 | Northeast Southeast South |
| [37]      | 1993 | Clinical report | Eumycetoma | 2 | Madurella mycetomatis | Bahia and Piauí (Northeast) |
| [26]      | 1994 | Clinical report + review | Actinomycetoma | 1 | Nocardia brasiliensis | Pará (North) |
| [28]      | 1995 | Clinical report | Actinomycetoma | 1 | Nocardia brasiliensis | Minas Gerais (Southeast) |
| [18]      | 1999 | Clinical report | Actinomycetoma | 1 | Unknown | São Paulo (Southeast) |
| [44]      | 1999 | Clinical report | Eumycetoma | 1 | Exophiala jeanselmei | Rio Grande do Sul (South) |
| [47]      | 1999 | Clinical report | Eumycetoma | 1 | Acremonium kiliense | Bahia (Northeast) |
| [78]      | 1999 | Clinical report | Eumycetoma | 1 | Madurella grisea | Rio Grande do Sul (South) |
| [51]      | 2002 | Clinical report | Eumycetoma | 1 | Fusarium solani | São Paulo (Southeast) |
| [29]      | 2004 | Clinical report | Actinomycetoma | 1 | Nocardia brasiliensis | Minas Gerais (Southeast) |

(Continued)
The mycetoma agents identified in our study are consistent with previous reports. In the actinomycetoma group, *Nocardia* spp., particularly *N. brasiliensis*, predominated and in the eumycetoma group, *Scedosporium apiospermum*. From 1980 to 2014, the main bacterial agents identified in Brazil were *Nocardia brasiliensis* [15,26,27–32], *Nocardia asteroides* [15,33], *Nocardia caviae* [34], *Actinomadura madurae* [13,35,36], *Actinomadura pelletieri* [14], and *Streptomyces somalienis* [15]. For eumycetoma were *Madurella mycetomatis* [15,25,37,38], *Madurella grisea* [39–45], *Acremonium falciforme* [46], *Acremonium kiliense* [47], *Scedosporium apiospermum* [12,18,48,49,50], *Fusarium solani* [51], *Exophiala jeaneselmi* [44,52,53] and *Aspergillus* sp. [12].

In our series of cases the diagnosis of mycetoma was made mainly by histopathological examination of affected tissues with visualization of the grains (approximately 91% of cases), while the isolation of the etiologic agent by culture was obtained in 66.6% of cases [15]. Implementation of molecular tools have recently demonstrated an improvement in the sensitivity and specificity in diagnosing mycetoma [16].

Radiography and ultrasonography were the most often used imaging because of their low cost and accessibility. Ultrasonography was crucial in identifying the presence of grains before diagnosis, during and after the therapeutic follow-up. Magnetic resonance imaging is the gold standard imaging method for mycetoma diagnosis and was important to delineate the involvement of internal structures and surgical planning [54]. CT scan was used if no bone involvement was detected by radiography.

### Table 4. (Continued)

| Reference | Year | Study | Classification | Number of cases | Etiologic agent | Region |
|-----------|------|-------|----------------|-----------------|-----------------|--------|
| [45]      | 2004 | Clinical report | Eumycetoma | 1 | **Madurella grisea** | Rondônia (North) |
| [30]      | 2007 | Case series | Actinomycetoma | 1 | *Nocardia brasiliensis* | Rio Grande do Sul (South) |
| [15]      | 2008 | Retrospective | Eumycetoma | 13 | *Madurella mycetomatis*—3, *Madurella grisea*—1, *Acremonium kiliense*—1, *Acremonium sp.*—1, *Culture negative*—7, *Nocardia brasiliensis*—3, *Nocardia asteroides*—1, *Streptomyces somalienis*—1, *Culture negative*—9 | Southeast Northeast, Southeast Northeast |
| [36]      | 2010 | Clinical report | Actinomycetoma | 1 | *Actinomadura madurae* | Paraiba (Northeast) |
| [34]**    | 2010 | Clinical report | Actinomycetoma | 1 | *Nocardia caviae* (*Nocardia otitidiscaviarum*) | Minas Gerais (Southeast) |
| [31]      | 2011 | Clinical report | Actinomycetoma | 1 | *Nocardia brasiliensis* | Northeast |
| [79]      | 2011 | Clinical report | Eumycetoma | 1 | - | São Paulo (Southeast) |
| [53]      | 2011 | Clinical report | Eumycetoma | 1 | *Exophiala jeaneselmei* | Paraná (South) |
| [38]**    | 2013 | Clinical report | Eumycetoma | 1 | *Madurella mycetomatis* | Ceará (Northeast) |
| [48]      | 2013 | Clinical report + review | Eumycetoma | 1 | *Scedosporium apiospermum* | Rio Grande do Sul (Southeast) |
| [32]      | 2014 | Clinical report | Actinomycetoma | 1 | *Nocardia brasiliensis* | São Paulo (Southeast) |
| [25]**    | 2014 | Clinical report | Eumycetoma | 1 | *Madurella mycetomatis* | Rio de Janeiro (Southeast) |

* Article with reference but not found.
** Article with doubt about the diagnostic proposed by the author because he has not reported the existence of grain.
*** Case report inside the study.
**** Review of the author.

doi:10.1371/journal.pntd.0005301.t004
Mycetoma treatment is challenging and usually requires long periods of drug therapy with or without surgical procedures (complete excision of the lesion, bone curettage, amputation) [1,5,8,10]. Itraconazole is the most common antifungal agent used for eumycetoma treatment [2]. Voriconazole and posaconazole have been indicated for refractory cases of mycetoma [58] primarily caused by S. apiospermum and Acremonium sp. [48,59–62]. They are expensive in underdeveloped countries and are not available in our institution. Isavuconazole and ravuconazole seem to be satisfactory against M. mycetomatis [63,64] but their effectiveness against other eumycetoma agents need to be investigated.

The first patient in this series of cases was evaluated in 1991, and because of this, the combination of drugs used was based on the available drugs at that time in our institution. The combined itraconazole/fluconazole, and itraconazole/terbinafina treatment in this study was chosen because of our good experience in treating extensive cutaneous lesions of chromoblastomycosis caused by Fonsecaea pedrosoi [55]. However, currently the itraconazole/fluconazole combination for mycetoma is not effective. Although liposomal amphotericin B are no longer recommended for first-line eumycetoma treatment, due to the high minimum inhibitory concentrations required for most eumycetoma agents [16,17,21,56,57], we tried to use only in one case due to clinical worsening during pregnancy, without success [25].

The recommended treatment for actinomycetoma is SMX/TMP as monotherapy or in combination with amikacin sulphate [10]. The association usually gives a cure rate above 90% [2, 65, 66]. Laboratory tests are required to assess possible adverse effects, as ototoxicity (cochlear lesions) and nephrotoxicity, which are permanent injuries, but are not progressive when treatment is suspended. In case 8 of Table 3 a combination with amikacin sulphate was used due to bone destruction. Amoxicillin and clavulanate are alternative drugs during pregnancy, for resistant cases or for patients with adverse effects from aminoglycoside [3]. Rifampicin can be used, but in Brazil it is reserved for tuberculosis and leprosy treatment, diseases with a high burden in our country. Minocycline and moxifloxacin are also treatment options for actinomycetoma [2, 67].

Surgery is indicated for small well localised lesions or in patients who are not responding to medical therapy or to reduce disease burden in massive lesions to allow a better response to medical therapy. [68]. Usually, actinomycetoma require less surgery management then eumycetoma [10]. Amputation are indicated for those patients with massive disease with no response to medical treatment or with massive bone destruction or in case with severe secondary bacterial infection not responding to medical treatment or with severe drug side-effects. [3]

Although our institution has provided all antimicrobials necessary for the treatment free of cost to all patients, the cure rate in this study was low, which reflects the difficulties in treating this disease. Besides the inconvenience to take pills every day for a long period, the total cost of mycetoma treatment is unaffordable for people living in poor regions where the disease commonly occurs. We suggest that the low rate of cure in our study is multifactorial, including the delay to obtain a correct diagnosis, and the scarcity of specialized surgical services with knowledge about this disease that allow the management of the most advanced cases. The postponement of diagnosis favours the occurrence of severe cases that are refractory to the treatment due to the low bioavailability and efficiency of some drugs in advanced lesions. Some patients of our study took more than a year to obtain a correct diagnosis and initiate adequate treatment.

In our cases, treatment dropouts was high and they were likely related to delayed clinical responses and the prolonged treatment times. Recurrence was also frequent [56] and prevailed in patients that had undergone surgery, especially in the eumycetoma group [38]. The reasons are unknown, but may be likely due to the existence of undiagnosed subclinical lesions fungal
defence mechanisms against antifungal drugs or incomplete surgical procedures. It is interesting to note that in case 2 (Table 2), the patient was considered clinically cured, but presented recurrence at the eighth year of follow-up [38]. In this case, however, exogenous reinfection cannot be ruled out. We did not observe a relationship between recurrence and a specific etiologic agent.

In rarely cases, mycetoma can spread along the lymphatics to the regional lymph node [6,68]. Few blood-spread mycetoma cases [7,16,69,70,71,72] and deaths related to the infection were reported [4,9,70], but they were not observed in our study.

Although with few cases, this study, highlights the wide spectrum of clinical manifestations of mycetoma, such as localized lesions, bone disease, worsening with pregnancy, recurrence and amputation cases. We also emphasize the challenges to treat and control this neglected disease. The accurate management of each case requires multiple experts including clinicians, surgeons, microbiologists, radiologists working together to assess the best therapeutic approach, which includes a prolonged treatment followed by a long follow up after achieving clinical cure. Rehabilitation is necessary in cases of deformity and amputation, unacceptable sequelae in the 21st century.

Acknowledgments

We are thankful for Marcel de Souza Borges Quintana, who helped during the statistic conclusions.

Author Contributions

Conceptualization: FMSS MCGG ACFdV.
Data curation: FMSS.
Formal analysis: FMSS MRL.
Investigation: FMSS MCGG ACGdv.
Methodology: FMSS MCGG ACFdV.
Project administration: FMSS.
Resources: MCdSL JMCdOC RdAP.
Software: FMSS MCGG ACFdV.
Supervision: BW.
Validation: FMSS BW DFSF.
Visualization: FMSS.
Writing – original draft: FMSS MCGG ACFdV.
Writing – review & editing: BW.

References

1. van de Sande WW. Global burden of human mycetoma: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2013 Nov 7; 7(11): e2550. doi: 10.1371/journal.pntd.0002550 PMID: 24244780
2. Welsh O, Al-Abdely HM, Salinas-Carmona MC, Fahal AH. Mycetoma Medical Therapy. PLoS Negl Trop Dis. 2014 Oct 16; 8(10): e3218. doi: 10.1371/journal.pntd.0003218 PMID: 25330342
3. Zein HA, Fahal AH, Mahgoub el S, El Hassan TA, Abdel-Rahman ME. Predictors of cure, amputation and follow-up dropout among patients with mycetoma seen at the Mycetoma Research Centre,
4. Mohamed NA, Fahal AH. Mycetoma pulmonary secondar ies from a gluteal eumycet oma: an unusual presenta tion. Plos Negl Trop Dis. 2016. 6; 10(10): e0004945. doi: 10.1371/jour nal.pntd.0004945 PMID: 22854685

5. Fahal AH. Review Mycetoma. Khartoum Medical Journal. 2011; 4(1): 514–523.

6. Freitas DF, Valle AC, da Silva MB, Campos DP, Lyra MR, de Souza RV, Veloso VG, Zanycop-Oliveira RM, Bastos FI, Galhardo MC. Sporotrichosis: an emerging neglected opportunistic infection in HIV-infected patients in Rio de Janeiro, Brazil. PLoS Negl Trop Dis. 2014 Aug 28; 8(8): e3110. doi: 10.1371/ journal.pntd.0003110 PMID: 25164745

7. Queiroz-Telles F, Nucci M, Colombo AL, Tobón A, Restrepo A. Mycoses of implantation in Latin America: an overview of epidemiology, clinical manifestations, diagnosis and treatment. Med Mycol. 2011; 49(3): 225–236. doi: 10.3109/13693786.2010.539631 PMID: 21128710

8. Fahal A, Mahgoub el S, El Hassan AM, Abdel-Rahman ME, Alshambaty Y, Hashim A, Hago A, Zijlstra EE. A new model for management of mycetoma in the Sudan. PLoS Negl Trop Dis. 2014; 8(10): e3271. doi: 10.1371/journal.pntd.0003271 PMID: 25356640

9. López-Martínez R, Méndez-Tovar LJ, Bonifaz A, Arenas R, Mayorga J et al. Update on the epidemiology of mycetoma in Mexico. A review of 3933 cases. GacMedMex. 2013; 149(5): 586–92.

10. Zijlstra E.E., Van De Sande Wendy W J, Welsh O., Mahgoub E. S., Goodfellow M., & Fahal A. H. Mycetoma: A unique neglected tropical disease. The Lancet Infectious Diseases. 2016; 16(1), 100–112. doi: 10.1016/S1473-3099(15)00359-X PMID: 26738840

11. Bonifaz A, Tirado-Sánchez A, Calderón L, Saúl A, Araiza J, Hernández M, González GM, Ponce RM. Mycetoma: experience of 482 cases in a single center in Mexico. PLoS Negl Trop Dis. 2014 Aug 21; 8(8): e3102. doi: 10.1371/journal.pntd.0003102 PMID: 25144462

12. Lacaz CS. Distribuição geográfica dos micetomas no Brasil. An Bras Dermatol. 1981; 56: 167–172.

13. Wanke NC, Wanke B, Cauuby MJ, Towersey L, Londero AT, Dias MF, Siqueira SP. Mycetoma due to Madurella mycetomatis. A report of 2 cases. Rev Inst Med Trop Sao Paulo. 1992; 34(4): 367–372. PMID: 1342096

14. Castro LG, Valente NY, Germano JA, Vaccari EM, da Silva Lacaz C. Mycetoma in an HIV-infected patient. Rev Hosp Clin Fac Med Sao Paulo. 1999; 54(5): 169–171. PMID: 10788840

15. Greenberg AK, Knapp J, Rom WN, Addrizzo-Harris DJ. Clinical presenta tion of pulmonary mycetoma in HIV-infected patients. Chest. 2002; 122(3): 886–892. PMID: 12226028

16. Gibson JN, Fulpo PP. Concurrent atazanavir and voriconazole in a patient with multidrug-resistant HIV and a mycetoma. AIDS. 2011.23; 25(16): 2054–2056. doi: 10.1097/QAD.0b013e32834babc9 PMID: 21997493

17. Moudgal VV, Sobel JD. Antifungal drugs in pregnancy: a review. Expert Opin Drug Saf. 2003; 2(5): 475–483. doi: 10.1517/14740338.2.5.475 PMID: 12946248

18. Lamont HF, Blogg HJ, Lamont RF. Safety of antimicrobial treatment during pregnancy: a current review of resistance, immunomodulation and teratogenicity. Expert Opin Drug Saf. 2014; 5: 1–13.

19. Butler DC, Heller MM, Murase JE. Safety of dermatologic medications in pregnancy and lactation: Part II. Lactation. J Am Acad Dermatol. 2014; 70(3): 417.e1–10; quiz 427. doi: 10.1016/j.jaad.2013.09.009 PMID: 24528912

20. Murase JE, Heller MM, Butler DC. Safety of dermatologic medications in pregnancy and lactation: Part I. Pregnancy. J Am Acad Dermatol. 2014; 70(3): 401.e1–14; quiz 415. doi: 10.1016/j.jaad.2013.09.010 PMID: 24528911

21. Sampaio FM, Gutierrez Galhardo MC, De Farias Cardoso R, de Oliveira Coelho JM, Rosandiski Lyra M, Francesconi do Valle AC. Eumycetoma on the Foot Caused by Madurella mycetomatis: Amputation After Significant Worsening During Pregnancy. Acta Derm Venereol. 2014 Sep 2.
26. Silva DB, Macedo C. Micetoma por Nocardia brasiliensis. An Bras Dermatol. 1994; 69(6): 485–487.
27. Mello Filho A, Proença NG, Rosa IP, Pereira WA. Micetoma due to Nocardia brasiliensis. Re-evaluation of twelve years after clinical cure. An Bras Dermatol. 1982; 57(4): 215–218.
28. Bulian AC Jr, Sena A-Z, Loiola ALP, Avelar JGL, Mattedi MGS, Vitória LC. Podal micetoma for Nocardia brasiliensis: a case report. An Bras Dermatol. 1995; 70(5): 447–449.
29. Motta RL, Vilela RV, Lambertiucci JR. Actinomyctoma caused by Nocardia brasiliensis. Rev Soc Bras Med Trop. 2004; 37(3): 287–288. PMID: 15330073
30. Chedd MB, Chedd MF, Porto NS, Severo CB, Severo LC. Nocardial infections: report of 22 cases. Rev Inst Med Trop Sao Paulo. 2007; 49(4): 239–246. PMID: 17823754
31. Cordeiro F, Bruno C, Reis C. Micetoma. An Bras Dermatol. 2011; 85(5):791. doi: 10.4269/ajtmh.2011.10-0637. PMID: 22049027
32. Sousa JM, Wachholz PA, Sette CS, Marques GF, Barreto JA. Micetomas caused by Nocardia brasiliensis in an immunocompetent patient. J Dtsch Dermatol Ges. 2014; 12(10): 903–905. doi: 10.1111/ddg.12331. PMID: 25135693
33. Sarac GD, Towersey L, Hay RJ, Londero AT, Martins Ede C, Amora AT, Reis KM, Mendonça AM, Estrella RR. Micetoma by Nocardia asteroides: a 9 year follow-up. Rev Inst Med Trop Sao Paulo. 1993; 35(2): 199–204. PMID: 8284606
34. Magalhães GM, Oliveira SC, Soares AC, Machado-Pinto J, de Resende MA. Micetoma caused by Nocardiaceae in the first Brazilian patient. Int J Dermatol. 2010; 49(1): 56–58. doi: 10.1111/j.1365-4632.2009.04262.x. PMID: 20466513
35. Lacaz CS, da Silva JG, Sabba LMB, de Melo NT, Heins-Vaccari EM, Calvis LA, Santos IO. Actinomycetoma por Actinomadura madurae with extensas lesões ósseas. An Bras Dermatol. 1984; 59(5): 244–248.
36. Dresch TF, de Magalhães TC, Piñeiro-Maceira J, Akiti T, Ramos-e-Silva M. Combined therapy for micetoma: medical and surgical. Dermatol Surg. 2010; 36(6): 952–954. doi: 10.1111/j.1524-4725.2009.01418.x. PMID: 20039916
37. Levites J, Acilchorne AOÁ, Gompertz OF. Fistulografia e cintilografia no estadiamento dos micetomas por Madurella mycetomatis. An Bras Dermatol. 1993; 68(4): 233–237.
38. Sampaio FM, Galhardo MC, Quintella LP, Souza PR, Coelho JM, Valle AC. Eumycetoma por Madurella mycetomatis with 30 years of evolution: therapeutic challenge. An Bras Dermatol. 2013; 88(6 Suppl 1): 82–84. doi: 10.1590/abd1806-4841.20132136. PMID: 24346887
39. Heins-Vaccari EM, Takahashi N, Oliveira NR, Lacaz CS, Porto E. Eumycetoma de grãos preto por Madurella grisea. Registro de um caso. Rev. Inst. Trop S Paulo. 1982; 29: 119–123.
40. Arruda Neto E, Pignatari ACC, Castello Filho A, Colombo AL, Longo JC, Camargo ZP. Eumycotic micetoma. Report of a case caused by Madurella grisea. Rev microbiol (S Paulo). 1989; 20: 485–500.
41. Belca W Junior, Cucé LC, Dias MC, Lacaz CS. Eumycetoma de grãos pretos por Madurella grisea. Rev. Inst Med Trop S Paulo. 1989; 31: 195–199.
42. Silva MRR, Fernandes OFL, Oliveira LM, Costa MB, Castro LC. Eumycetoma por Madurella grisea. Relato de Caso. Rev soc bras medic trop. 1991; 24: 51–54.
43. Machado LAP, Rivitti MCM, Cucé LC, Salebian A, Lacaz CS, Heins-Vaccari EM, Belca W Junior, Takahashi N. Eumycetoma de grãos pretos por Madurella grisea. Registro de dois casos. Rev Inst. Med trop São Paulo.1992; 34(6): 569–580.
44. Severo LC, Oliveira FM, Vettorato G, Londero AT. Micetoma caused by Exophiala jeaneselmei. Report of a case successfully treated with itraconazole and review of the literature. Rev Iberoam Micol. 1999; 16(1): 57–59. PMID: 18473595
45. Vilela R, Duarte OM, Rosa CA, Castro JG, Lyon SJ, Motta RL, Moura AC. A case of eumycetoma due to Madurella grisea in northern Brazil. Mycopathologia. 2004; 158(4): 415–418. doi: 10.1007/s11046-004-2844-y. PMID: 15630550
46. Zaitz C, Lacaz CS, Salebian A, Ruiz LR, Heins-Vaccari EM, de Melo NT. Eumycetoma podal por Acremonium falciforme. Registro de um caso. An Bras Dermatol. 1988; 63(4): 413–418.
47. Lacaz CS, Pereira AD, Castro LGM, Nunes RS, Heins-Vaccari EM, de Freitas RS, Arriagada GLH. Eumycetoma podal por Acremonium kiliani: registro de um caso. An bras dermatol. 1999; 74(6): 591–595.
48. Oliveira FM, Unis G, Hochhegger B, Severo LC. Scedosporium apiospermum eumycetoma successfully treated with oral voriconazole: report of a case and review of the Brazilian reports on scedosporiosis. Rev Inst Med Trop Sao Paulo. 2013; 55(2): 121–123. PMID: 23563766
49. da Rocha OM, Lacaz Cda S, Porto E, Heins EM, Schaf S, Hirose-Pastor E, Cossermelli W. Articular mycetoma caused by Petriellidium boydii. Report of a case. Rev Inst Med Trop Sao Paulo. 1980; 22(1): 24–29. PMID: 7192011
50. Purchio A, Gambale W, Paula CR, Yamamura I, Cavalcante ASB. Mycetoma of the arm by Petriellidi um boydii. A case report. J bras dermatol. 1981; 56(4): 281–284.

51. Tomimori-Yamashita J, Ogawa MM, Hirata SH, Fschiman O, Michalany NS, Yamashita HK, Alchone M. Mycetoma caused by Fusarium solani with osteolytic lesions on the hand: case report. Mycopathologia. 2002; 153(1): 11–14. PMID: 11913759

52. Van de Sande WW, Maghoub el S, Fahal AH, Goodfellow M, Welsh O, Zijlstra E. The mycetoma knowledge gap: identification of research priorities. PLoS Negl Trop Dis. 2014; 27; 8(3): e2667. doi: 10.1371/journal.pntd.0002667 PMID: 24675533

53. Van Belkum A, Fahal AH, van de Sande WW. In vitro susceptibility of Madurella mycetomatis to posaconazole and terbinafine. Antimicrob Agents Chemother. 2011; 55(4): 1771–1773. doi: 10.1128/AAC.01045-10 PMID: 2163050

54. Ahmed SA, Kloezen W, Duncanson F, Zijlstra EE, de Hoog GS, Fahal AH, van de Sande WW. Madurella mycetomatis is highly susceptible to ravuconazole. PLoS Negl Trop Dis. 2014.19 ; 8(6): e2942. doi: 10.1371/journal.pntd.0002942 PMID: 24945848

55. Welsh O, Saucedo E, Gonzalez J, Ocampo J. Amikacin alone and in combination with trimethoprim-sulfamethoxazole in the treatment of actinomycotic mycetoma. J Am Acad Dermatol. 1987; 17(3): 443–448. PMID: 3039890

56. Gomez-Flores A, Welsh O, Said-Fernández S, Lozano-Garza G, Tavarez-Alejandro RE, Vera-Cabrera L. In vitro and in vivo activities of antimicrobials against Nocardia brasiliensis. Antimicrob Agents Chemother. 2004; 48(3): 832–837. doi: 10.1128/AAC.48.3.832-837.2004 PMID: 14982772

57. Antunes J, Pacheco D, Travassos R, Sequeira H, Filipe P, Marques MS. Actinomycetoma of the chest wall associated with Nocardia nova after reconstructive surgery. Dermatol Online J. 2012; 18(1):4. PMID: 22301041

58. Suleiman SH, Wadaella el S, Fahal AH. The Surgical Treatment of Mycetoma. PLoS Negl Trop Dis. 2016; 23; 10(6):e0004690. doi: 10.1371/journal.pntd.0004690 PMID: 27336736

59. Fahal AH. Mycetoma thorn on the flesh. Review article. Trans R Soc Trop Med Hyg. 2004; 98 (1) 3–11. PMID: 14702833

60. Ahmed AA, van de Sande WW, Fahal A, Bakker-Woudenberg I, Verbrugh H et al. Management of mycetoma: major challenge in tropical mycoses with limited international recognition. Curr Opin Infect Dis. 2007; 20(2):146–51. doi: 10.1097/QOC.0b013e32803d38fe PMID: 17496572
71. Mohamed el SW, Seif El Din N, Fahal AH. Multiple Mycetoma Lung Secondaries from Knee Eumycetoma: An Unusual Complication. Plos Negl Trop Dis. 2016; 10(7): e0004735. doi: 10.1371/journal.pntd.0004735 PMID: 27442512

72. Arbab MA, el Hag IA, Abdul Gadir AF, Siddik H el-R. Intraspinal mycetoma: report of two cases. Am J Trop Med Hyg. 1997; 56 (1):27–29. PMID: 9063356

73. Talhari Sinêsio, Gadelha Alcidart a Dos Reis, Cunha Maria Da Graça Souza, Fernandes Gilberto, Paes Marcilene Gomes et al. Deep mycosis in Amazonia—Study of diagnosed cases in Manaus—Amazon as State, from 1973 to 1978. An Bras Dermatol. 1980; 55(3): 133–136.

74. Londero AT, Ramos CD, Matte SW. Micetomas actinomicoticos no Rio Grande do Sul: relato de quatro casos. Mem. Inst. Oswaldo Cruz. 1986; 81(1): 73–77

75. Talhari S, Cunha MG, Schettini AP, Talhari AC. Deep mycoses in Amazon region. 1988; 27(7): 481–484.

76. Mapurunga ACP, Gonçalves HMG, Silva JB, Cabral SESX, Diógenes MJD. Micoses profundas no Ceará. Estudo dos casos diagnosticados no Hospital das Clínicas da Universidade Federal do Ceará (1983 a 1988). An bras dermatol. 1990; 65(3): 117–118.

77. Silva MRR, Fernandes OFL, Silva HM. Agentes etiológicos de micetoma ocorridos em Goiania-Goiás. Rev microbiol (S Paulo). 1991; 22: 39–41.

78. Severo LC, Vetoratto G, Oliveira Fde M, Londero AT. Eumycetoma by Madurella grisea. Report of the first case observed in the southern Brazilian region. Rev Inst Med Trop Sao Paulo. 1999; 41(2): 139–142. PMID: 10413963

79. Nai GA, Stuani ML, Stuani LA. Oral cavity eumycetoma. Rev Inst Med Trop Sao Paulo. 2011; 53(3): 165–168. PMID: 21755239