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GENERAL REVIEW/REVUE GÉNÉRALE

Chromoblastomycosis and sporotrichosis, two endemic but neglected fungal infections in Madagascar

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Summary Chromoblastomycosis and sporotrichosis are endemic fungal infections of tropical and subtropical regions, including Madagascar. The causal fungi develop in the soil or on plants and infect humans through wounds, either directly (wounding by the plant, through thorns, for example), or through the contact of an existing wound with contaminated soil. For this reason, the lesions predominantly occur on the limbs, and these fungi principally infect people working

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outside with bare hands and/or feet. The subcutaneous lesions of chromoblastomycosis are initially nodular, subsequently becoming warty, tumoral, cauliflower-like and pruriginous, which promotes dissemination. The chronic nature of the infection and its progression over long periods lead to highly disabling lesions in essentially rural and agricultural populations. The lesions of sporotrichosis are also nodular, but more ulcerous, and they form an extended chain following the route of the lymph vessels. Pus, squamous or skin biopsy specimens are used for the mycological examination of these mycoses. Treatment depends on the severity and form of the lesions and is based on antifungal drugs sometimes combined with physical methods. There has been no study of these infections for more than two decades in Madagascar, despite the large numbers of cases seen by doctors in all parts of the island. The nature, diversity and distribution of the plants responsible for contamination have not been described in Madagascar. In this review, we described these two endemic mycoses in terms of their epidemiological, mycological, clinical and therapeutic characteristics, focusing particularly on Madagascar, which is one of the leading foci of these two infections worldwide.

Introduction

Chromomycosis or chromoblastomycosis (CBM) and sporotrichosis (SPT) are chronic subcutaneous or cutaneolymphatic infections found mostly in tropical and subtropical regions [1—4]. Studies carried out by the Institut Pasteur of Madagascar between 1955 and 1994 provided an inventory of the number of cases of CBM and identified this country as the leading focus of this mycosis worldwide. Mean incidence was estimated at about 1/200,000 inhabitants at the time [5]. Since 1994, no further studies on CBM have been performed in Madagascar and no new data have been obtained to update the epidemiological situation [6]. CBM is usually caused by dematiaceous fungi (also known as black yeasts), principally *Fonsecaea pedrosoi* and *Cladophialophora carrionii*. The causal agent of SPT is *Sporothrix schenckii*, a dimorphic hyphomycete [4,7]. Only sporadic cases have been reported in Madagascar, due to the absence of specific surveillance. SPT may be polymorphic and disseminated, with pulmonary forms.

These fungal infections are typical of so-called ''mycoses of implantation or inoculation'', because they are linked to traumatic inoculation with the fungus through wounding by the plant or through soil contamination of an existing wound [8,9]. The chronic course and long duration of lesions may result in considerable disability in the absence of treatment. Due to the large number of cases worldwide, their geographic distribution, professional origin, preponderance among the poorest populations in the world and refractory nature, these infections are candidates for inclusion in the WHO list of neglected tropical diseases. In Madagascar, it is rarely possible to confirm clinically suspected infections or to identify the fungal species responsible, because health structures are underequipped and resources are limited. Given the large number of cases currently being seen by doctors throughout the island, the prevalence of these infections appears to be largely underestimated. In this review, we describe these two fungal infections, focusing on the most recent studies and the situation in Madagascar. We also report preliminary results for the first 28 months of a prospective study currently underway, involving the recruitment of patients with suspect lesions. The confirmation of infection by mycological and molecular biology methods in this study suggests that these two infections remain highly prevalent in this country.

Chromoblastomycosis

CBM is a phaeohyphomycosis, but it has a number of clinical and diagnostic features that are pathognomonic and distinguish it from the other diseases of this group, such as mycetoma. Indeed, CBM is a chronic fungal infection of the subcutaneous tissues characterized by warty hyperkeratosic plaques and the presence of fumagoid cells on microscopic examination of squamous samples. The causal fungi develop on living plants or on decomposing plant material in the soils of tropical and subtropical regions.

Pathogenic agents

The pathogenic agents responsible for CBM are fungi from the phylum Ascomycetes, mostly from the order Chaetotyriales, and family Herpotrichiellaceae. This family includes the black yeasts of the genus *Exophiala* and dematiaceous filamentous fungi, including the causal agents of CBM: *Fonsecaea* spp., *Phialophora verrucosa*, *Cladophialophora carrionii* and *Rhinocladiella aquaspersa* [10—12]. *F. pedrosoi* is particularly abundant in the Amazon rainforest in Brazil [13]. It was first isolated by Alexandrino Pedroso in 1911 [14] and was subsequently described as *Hormodendrum pedrosoi* by Brumpt in 1922. The genus *Fonsecaea* was established in 1936, by Negroni [15]. Several species of this genus other than *F. pedrosoi* have been implicated in CBM: *F. monophora*, *F. nubica* and *F. pugnacius*. Another species, *F. compacta*, has been reported to be a morphological variant of *F. pedrosoi* [16,17]. A recent study described two new species isolated from thorny plants: *F. erecta* and *F. minima* [18].

P. verrucosa was first described by Medlar in 1915 [10]. Several authors have described this fungus as a causal agent of CBM [19] and of phaeohyphomycotic cysts [20].

C. carrionii was first identified in the arid regions of South America, Africa and Australia. Trejos described it under the

Figure 1 Thorny plants of *Solanum erythrecanthum* (A) and *Rubus rosifolius* (B) growing in the north-north east region of Madagascar.

name of *Cladosporium carrionii* [21]. Recent taxonomic studies of pathogenic species have led to a change in the name of the genus from *Cladosporium* to *Cladophialophora* [22].

R. aquaspersa was described by Borelli, Schell et al. in 1972 and has been reported to be responsible for rare cases of warty infections of the skin in Mexico and Brazil [23].

In the environment, the fungi responsible for CBM are found in soil organic matter, water and on plants [24,25]. *F. pedrosoi* has been isolated principally from decomposing plant matter and soil [26]. According to Salgado et al., *Mimosa pudica*, a plant with thorny stems, is a source of *F. pedrosoi* infections in Brazil [25]. However, all these studies were based on morphological rather than molecular identification techniques. *C. carrionii* was reported to be present on cactus thorns and fragments, but this finding was subsequently corrected by de Hoog et al., who identified the species present as *C. yegresii* [27,28]. Another potential source of the dematiaceous agents responsible for CBM is the Palmaceae family (palms), which is widely exploited in tropical zones [12,29]. It has also been shown that the identification of fungi as *F. pedrosoi* and *Cladophialophora* sp. on the basis of their morphological features in the environment, and on the basis of physiological and fermentation tests in the laboratory, may not be confirmed in molecular biology tests. Caution is therefore required when attributing responsibility to a particular plant species as the source of infection [18].

Epidemiology and geographic distribution

CBM is present mostly in the tropical and subtropical regions of America, Asia and Africa [12]. High prevalences have been reported in Mexico, Cuba, Venezuela, Colombia and Brazil and in Central, Sub-Saharan and North Africa. In his review, Queiroz-Telles described cases in South Africa and Algeria [3,12,30—35]. In Asia, CBM is most frequent in Japan, Sri Lanka, India and China [1,36—38].

In Madagascar, Guillet and Radaody-Ralarosy reported the first probable case of CBM in 1940 [39]. The arrival of E.R. Brygoo at the Institut Pasteur of Madagascar in 1950 made it possible to authenticate new cases of CBM [11,39] caused by *F. pedrosoi* and *C. carrionii*. Studies carried out at the Institut Pasteur between 1955 and 1994 identified 1343 cases of CBM, making this country the leading focus of this subcutaneous mycosis worldwide, with an estimated prevalence of 1/200,000 inhabitants at the time. The epidemiological data for this disease in Madagascar have not been updated since 1994 [5].

Most of the infected patients are men working in agriculture [5,6,25]. Most are involved in the cropping, by hand, of vanilla, coffee or sugar cane, but some are involved in husbandry and the rearing of livestock (e.g. pigs or zebu cattle) close to plantations. These farmers also engage in woodcutting and charcoal-producing activities. Due to the influence of the trade winds to the east and monsoons to the north, Madagascar has two distinctly different climates: a humid tropical climate, in which *F. pedrosoi* is found (in the east, north and north-west) and a semi-arid climate in which *C. carrionii* is found (in the south) [5,40]. The humid climate of the north and east is marked by a mean annual precipitation of more than 1500 mm and a mean temperature of more than 15 °C, conditions favoring the multiplication of *F. pedrosoi*. There is a considerable natural diversity of plant species in this region, including orchids (Orchidaceae) and palms (Arecaceae). In the northeast, vanilla (*Vanilla planifolia*) plantations, woody perfume tree forests, bamboo (Bambuseae), palms and trees of the genus *Tambourissa* predominate. Thorny plants responsible for transferring the fungi to humans, such as ''sakoa'' (*Solanum macrocarpum*) and ''angivibe'' (*Solaneum erythrecanthum*) and brambles or ''voarô'' (*Rubus rosifolius*) are also abundant in this region (Fig. 1).

By contrast, in the south, precipitation is much lower, at 300 to 600 mm, with a mean temperature of more than 20 °C throughout the year. Eight to nine months per year are dry. In these conditions, *C. carrionii* predominates. This thorn forest zone is characterized by a forest of thorny plants of the Didieraceae, together with euphorbias and plants from the Apocyanaceae. It also contains sisal (*Agave sisalana* [41]), which is used for the manufacture of rope, eucalyptus

(*Eucalyptus*), which is used for charcoal production, and cacti (*Alluaudia procera*) used in construction materials [6].

In 2012, the Charles Mérieux Infectiology Centre (CMIC) of the University of Antananarivo and the dermatology department of Joseph Raseta Befelatanana University Hospital Antananarivo, together with Grenoble-Alpes University, initiated a collaborative study designed to update prevalence data, and to describe the distributions of cases and causal agents in Madagascar. Preliminary results for the first 28 months of our prospective study, for the period from March 2013 to June 2015 [42], suggest that the prevalence of CBM is high, and confirm that *F. pedrosoi* and *C. carrionii* are the principal causal species. Eight cases of CBM were confirmed among the 55 patients (15%) included on the basis of suspicious lesions at a dermatology consultation in the capital or at district hospitals. The species *F. pedrosoi* (7 cases) in the south and southeast and *C. carrionii* (1 case) were identified by microscopy, and confirmed by sequencing.

Clinical presentation

Following inoculation, a primary lesion appears as a small papule that develops and forms papulosquamous plaques or becomes nodular within a few months. The proliferation of the fungus may then result in a warty florid appearance, or lead to the centrifugal extension of plaques or the formation of large plaques or extended circumferential lesions of the affected limb at advanced stages.

Plaque forms are generally violet in color, with a well-delimited raised border. The central part of the plaque may have healed and may present an infiltrated, squamous appearance (Fig. 2A). In nodular forms, the nodules may be isolated or multiple and may resemble large soft warts (Fig. 2B). The warty forms are characterized by pink pimples with a typical ''cauliflower'' appearance (Fig. 2C).

In most cases, the CBM lesions are localised on the lower limbs, particularly on the dorsal surface of the feet, the ankles and the legs [43]. These parts of the body are those most exposed to contamination from plants or soil. Surprisingly, lesions do not occur on the soles of the feet, even though the subjects at risk walk and work barefoot. The upper limbs and the buttocks are also frequently affected. Lesions on the ears, face and trunk are less common [44]. The lesion progresses slowly, over a period of two to 20 years. If treatment is absent or insufficient, the lesion extends and becomes highly disabling due to the development of elephantiasis-type oedemas or superinfections. Itching and scratching of the lesion favor dissemination. The lesions may vary in appearance, but no distinct forms associated with individual species (*C. carrionii* or *F. pedrosoi*) have been described [39]. Malignant transformation into epidermoid carcinoma is rare and concerns long-standing, advanced lesions [12,45].

Diagnosis

Direct microscopic examination of specimens

Clinical specimens (biopsy, squamous or pus specimens) are examined in chlorazol black solution or in a potassium solution (10 to 40% KOH). The presence of characteristic muriform cells in superficial samples is used to confirm the diagnosis of CBM. These cells are grouped into compartmented ''clusters'' of 4 to 12 μm in diameter, which are brown in color and have a thick wall. These clusters consist of a combination of skin cell debris, dried blood and fungal structures. Some cells may display the first signs of filamentation but should nevertheless be distinguished from the filamentous forms encountered in cases of disseminated phaeohyphomycosis [12]. These elements have also been described in microabscesses, dermal tissues and infected epidermis (Fig. 3).

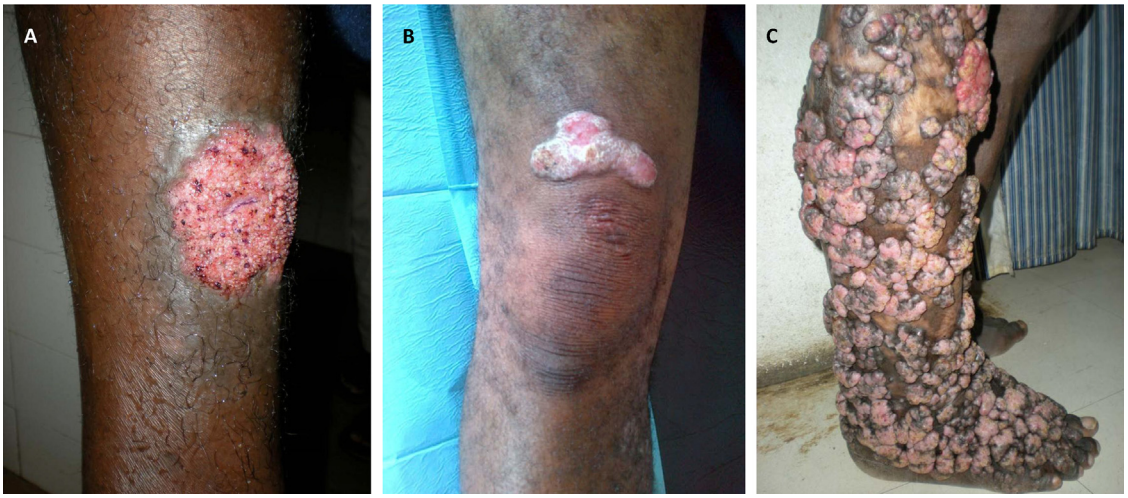

Figure 2 Clinical appearance of chromoblastomycosis on the lower limb. Single lesion, 8 cm in diameter, tumorous, scabby, keratosic, weeping, scaly and pruriginous, after more than two years of progression (A); single nodular lesion with a raised border, pink with blackish spots, after seven years of progression (B); papulosquamous plaque lesions, breaking out into ''cauliflower-shaped'' pimples, after more than four years of progression (C).

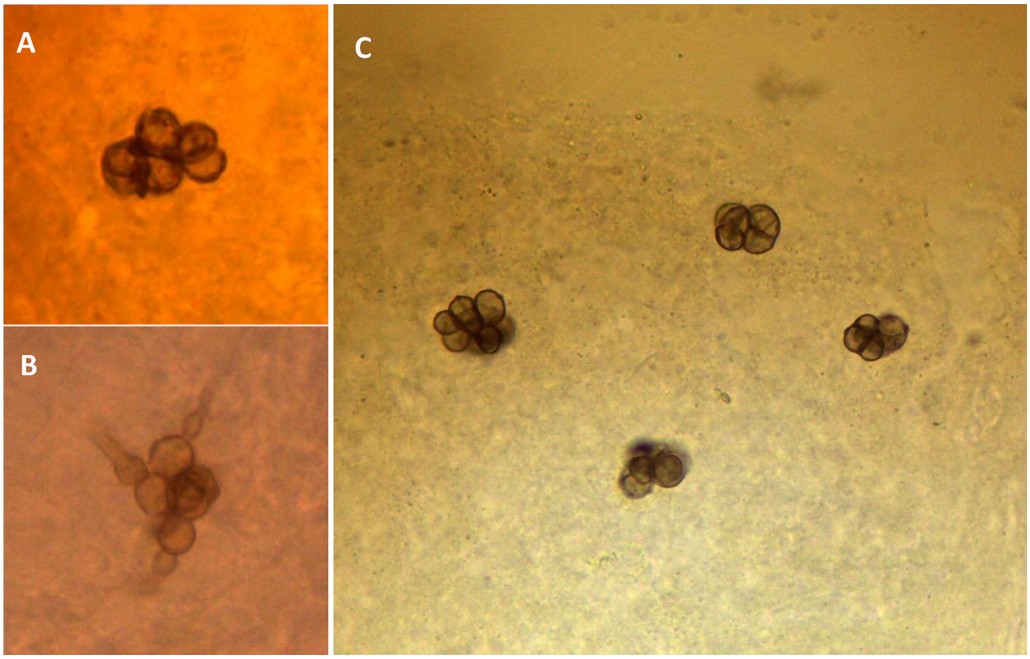

Figure 3 Muriform cells present in skin biopsy specimens from patients with chromoblastomycosis caused by *Cladophialophora carrionii* (A) and *Fonsecaea pedrosoi* (B, C). Photomicrographs (× 400 magnification) obtained after staining with chlorazol black and mounting of the specimen on a slide with a coverslip.

Histology

Histological sections of the epidermis display hyperplastic lesions and papillomatous proliferation. Hyperacanthosis is observed much more frequently than dyskeratosis. Muriform cells are present in a granulomatous reaction involving lymphocytes, plasmocytes and inflammatory cells, including giant Langhans cells.

Culture

The detection of muriform cells can be used as the basis of the diagnosis, but isolation of the fungus by culture is indispensable for identification of the causal agent and subsequent treatment, because *F. pedrosoi* may be less susceptible to antifungal drugs than *C. carrionii* [46,47]. The causal agents of CBM grow on the usual culture media, but their growth is slow, and up to six weeks of incubation at 27 to 30 °C may be required [1,13]. Selective media containing chloramphenicol and/or cycloheximide do not inhibit their growth.

Cladophialophora spp. form powdery black or olive green colonies. Under the microscope, the conidiophores are long, branched or unbranched and bear long chains of smooth-walled or slightly rough conidia (Fig. 4A, C). *Fonsecaea* spp. form very hard black colonies. Under the microscope, septate hyphae, cylindrical conidiophores, short phialides with and without collars, denticulate forms and fine-walled conidia are visible (Fig. 4B, D).

Microscopic examination can provide identification to the genus level, but sequencing of the ITS regions of rDNA is essential for identification to species level.

In the ongoing prospective study, diagnosis is confirmed by direct examination and culture at the CMIC and histological examination at Joseph Raseta Befelatanana University Hospital, Antananarivo. Molecular methods (PCR followed by sequencing and specific PCR) are performed at the CMIC and at Grenoble-Alpes University.

Treatment

Depending on disease severity and the form of the lesions, treatment may be based on antifungal drugs, surgery, laser vaporization, thermotherapy or cryotherapy. However, other than for lesions that have progressed very little and are excised very early by surgery, complete cure, with sterilization of the lesions, is rarely possible. The refractory nature of this infection generally renders very long-term treatment necessary.

Antifungal treatments

The susceptibility of the strains responsible for CBM to antifungal drugs in vitro is only partially predictive of treatment success. Such analyses provide no information about the efficacy of these drugs against the fungal forms present in the muriform cells, which are probably not very accessible to these molecules [12].

With this caveat concerning the interpretation of in vitro tests, most systemic antifungal drugs are effective against *Fonsecaea* spp. and *Cladophialophora* spp., which are highly susceptible to triazoles, such as itraconazole, posaconazole, voriconazole and isavuconazole, but not to fluconazole [47—50]. Posaconazole seems to be the most active of the triazoles against these fungi. *F. pedrosoi* has proved less susceptible than *F. nubica* and *F. monophora* to voriconazole and isavuconazole [46]. Terbinafine, a non-triazole compound, is also

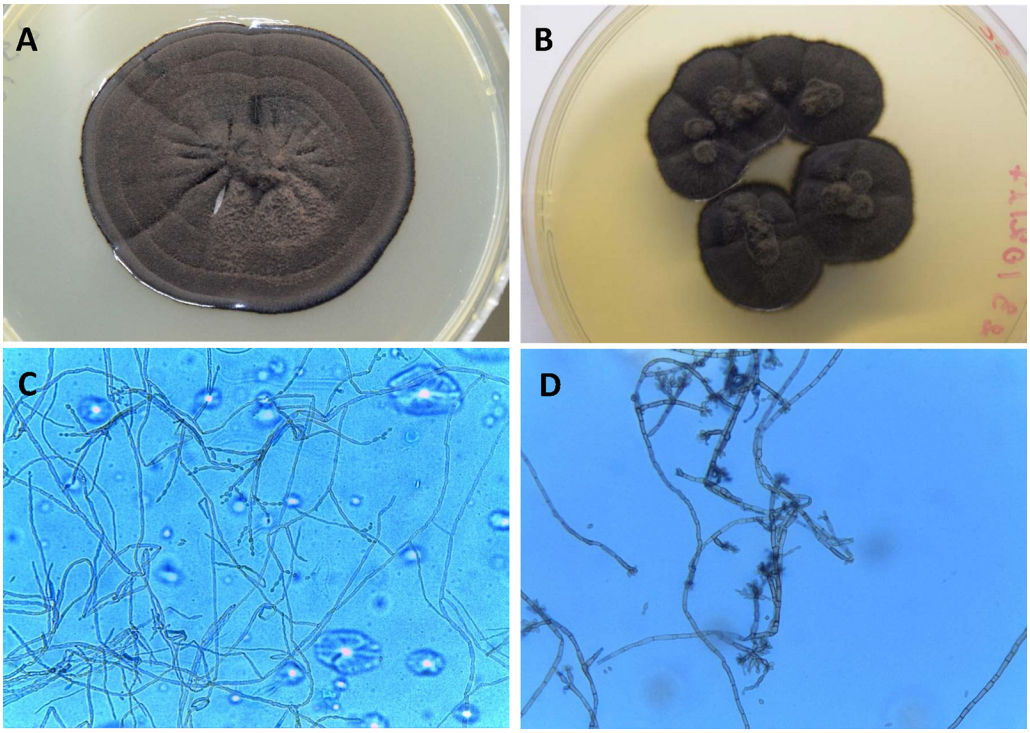

Figure 4 Macroscopic appearance of cultured colonies of *Cladophialophora carrionii*, furry and dark grey in color (A); and of *Fonsecaea pedrosoi*, fluffy and black (B) after 28 days of incubation at 30 °C on Sabouraud agar supplemented with chloramphenicol. Photomicrographs (× 400) obtained after the staining of cultured colonies of *Cladophialophora carrionii* with lactophenol blue. Note the dark, septate filaments bearing ovoid spores in short chains (C), and the *Fonsecaea pedrosoi* colonies with dark, septate filaments and denticulate forms (D).

active against the causal agents of CBM. Minimal inhibitory concentrations are higher for amphotericin B and echinocandins.

Itraconazole is the most widely used treatment [51], with a treatment success rate of 15 to 80%, depending on the study considered [12,30,51—53]. However, this drug seems to be less effective against *F. pedrosoi* than against *C. carrionii*. Doses of 200 to 400 mg/day are recommended. Treatment for two to six months is recommended for non-progressive lesions, whereas treatment for six to 12 months may be required for very advanced lesions. Itraconazole is sometimes used in combination with 5-fluorocytosine or surgery [54]. Posaconazole, at a recommended dose of 800 mg/day, is effective and has a success rate of 82% in refractory cases [55]. Unfortunately, too few clinical studies have yet been performed with the new triazoles, such as the gastroresistant form of posaconazole and isavuconazole, but these molecules appear to be highly active in vitro [46]. Together with itraconazole, terbinafine is the most widely used antifungal drug, at a dose of 500 mg/day for six to 12 months [53,56]. Its clinical efficacy is similar to that of itraconazole and P. Esterre demonstrated the utility of this drug for treating CBM in Madagascar [57], with a success rate of 75% for 42 treated cases [58].

Immunomodulatory treatment

The use of imiquimod, an agonist of toll-like receptors (TLRs), as a 5% topical application, has been shown to accelerate lesion regression after a transient phase of inflammatory exacerbation. This treatment, applied five times per week, has been tested in four patients, with satisfactory results [59].

Physical methods

Surgery and cauterization are systematically recommended for new single lesions. The dissemination and persistence of the fungus in the deep dermis often lead to recurrence.

As the fungus is unable to grow in vitro at temperatures above 40 °C, thermotherapy is often used, with the application of a constant source of heat (40 to 43 °C) on the infected skin. A series of cases successfully treated by this approach has been reported [60,61]. Other studies have shown thermotherapy to be effective in combination with posaconazole or terbinafine [54,57].

Cryotherapy is based on the use of a liquid nitrogen spray. This method yields better results when used in combination with antifungal drugs [13,46]. Cryotherapy is recommended for the treatment of small-localised lesions, to reduce scarring.

Laser vaporisation is a very promising method. The combination of this method with thermotherapy has been shown to be effective against recurrences [62].

CBM is currently being added to the WHO list of neglected tropical diseases, and Madagascar and Brazil actively participated in the request for its addition to this list. A national program to combat CBM in Madagascar is currently being

developed. The choice of treatment is currently dictated by access to drugs, with only itraconazole and terbinafine available, but at too high costs for the general public. In our study, the treatments currently used in Madagascar are delivered to the patients free-of-charge. The patients receive 200 mg itraconazole per day. Efficacy is variable, with very good treatment responses sometimes obtained as early as the second month of treatment, whereas other patients show no signs of a clear improvement after eight months of itraconazole treatment, leading to the addition of terbinafine to the treatment regimen, at a dose of 100 mg/day.

Sporotrichosis

SPT is a mycosis of animals and humans. It may be subacute or chronic and it develops following traumatic inoculation of the dermis with spores or mycelial fragments of the fungus *Sporothrix schenckii*. Its typical clinical presentation is a cutaneous/lymphatic form with chains of nodular ulcerous/scabby lesions on a limb.

Pathogenic agent

S. schenckii was first described by Benjamen Schenck in 1896, from a sample collected from the arm of a patient at Johns Hopkins Hospital in Baltimore. *S. schenckii* belongs to phylum Ascomycetes, class Pyrenomycetes, order Ophiostomatales and family Ophiostomataceae, which includes pathogenic fungi of trees in temperate regions [4,63].

However, SPT may also be caused by agents other than *S. schenckii*. Marimon et al. [64] performed genotypic and phenotypic analyses leading to the description of four new species of the *Sporothrix* complex: the cosmopolitan species *S. globosa* [65]; *S. brasiliensis* in Brazil [64,66]; *S. mexicana*, found only in Mexico [64] and *S. luriei*, formerly known as *S. schenckii* var. *luriei* [67]. Meyr and coworkers have also recently described three other species of *Sporothrix* present in the environment: *S. stylites*, *S. humicola* and *S. lignivora*. *S. humicola* has been described as the environmental form of *S. schenckii* [4,68].

S. schenckii is a dimorphic fungus, with two different morphological appearances depending on temperature. When cultured at 25 °C on standard medium (Sabouraud agar supplemented with chloramphenicol), *S. schenckii* adopts a filamentous form with diverse fruiting structures, including hyaline conidia and pigmented spores. When this fungus is cultured at 37 °C on blood agar, globular or elongated yeast-like forms are observed [69]. As human body temperature is about 37 °C, this fungus develops in the yeast form after entering the body via the skin.

Epidemiology and geographic distribution

SPT is a widespread infection, but it is particularly prevalent in the tropical and subtropical zones of Brazil, India, Mexico, the United States, Japan and South Africa [70—75]. It is rare in Europe, but there has been no epidemiological study of its prevalence. Only sporadic cases have been reported in France [76] and Italy [77].

SPT principally affects rural populations in frequent direct contact with the environment (soil and plants), particularly those working with bare hands and feet, such as farmers (whether involved in agronomy or husbandry), carpenters and charcoal producers. However, it is also found in people simply walking outside without shoes, including students and traders.

Animal scratches and bites have also been reported as another mode of *S. schenckii* transmission to humans. The animals most frequently recognised as responsible for this transmission are armadillos, squirrels, dogs and cats [4,78,79]. One epidemiological study reported that 156 of 178 cases in human concerned people in contact with infected cats and that 97 of these cases followed scratches or bites [70]. *S. schenckii* is present on the claws and in the buccal cavity of cats [80]. Farmers and veterinary surgeons are the individuals most exposed to this mode of transmission [4,81].

There are no epidemiological data concerning this mycosis in Madagascar, despite the large number of cases seen in hospitals and in consultations with dermatologists and infectious biology specialists. In 2007, the dermatology department of Antananarivo University Hospital confirmed a series of cases, one of which was dealt with in a detailed publication [82,83]. A single case was identified in the review by Lima Barros et al. published in 2011, for which the date and region were not specified [4]. It is therefore not impossible that this case and the case published in 2007 are one and the same.

The importance and history of sporotrichosis in Madagascar are thus difficult to evaluate and this infection has probably been largely neglected due to a lack of diagnostic tools. In our above mentioned prospective study, 18 cases (33%) of sporotrichosis have been confirmed by culture and molecular biology methods. *S. schenckii* is the only species to have been identified in these cases to date. The prevalence of sporotrichosis in Madagascar, which has never before been estimated, appears to be even greater than that of CBM. These results remain to be confirmed.

Distribution of cases according to climate and vegetation

In the environment, *S. schenckii* is found in the soil, on plants, on sphagnum moss, hay and tree bark and debris [73,84]. It has also been detected in air, water and other matter contaminated with soil [85]. According to Vásquez-del-Mercado et al., *S. schenckii* is more frequent in zones in which the temperature varies between 15 and 25 °C, with a relative humidity of 90% [7].

Clinical presentation

The principal clinical forms of SPT are the cutaneous-lymphatic form and the pulmonary and disseminated form.

Cutaneous-lymphatic form

This is the most frequent form. The SPT lesions are mostly located on the hands, feet and legs, but may also be found on the face, particularly in children [86].

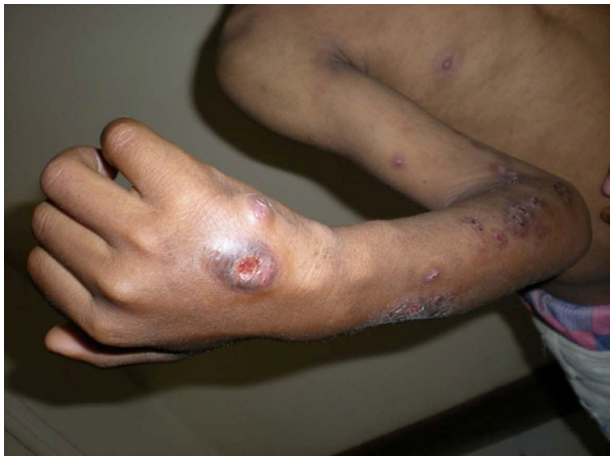

Figure 5 Clinical appearance of sporotrichosis in the form of scabby, ulcerated lesions following the course of the lymph vessels associated with signs of inflammation (pain, redness) after progression for about a month.

The incubation phase may last from a few days to a month. The patients then begin to feel a sensation of discomfort at the site of inoculation. The reddish papular lesion gradually increases in size, becoming nodular, warty or ulcerated after about two weeks, but remaining painless: this lesion is known as the sporotrichosic or inoculation chancre. In some cases, there is already a satellite adenopathy at this stage. The lesion may remain stable for several weeks or months and may even disappear spontaneously [29]. It sometimes extends along the route of the lymph vessels, forming chains of nodules in the hypoderm [87]. These lesions are ulcerous or pimple-like in appearance and are pink or violet, sometimes tending towards black, in color [7]. Ulcerated, inflammatory and scabby nodular lesions represent progression and are found in chains along the route of the lymph vessels of the affected limb (Fig. 5). These lesions have a depressed border and an inflammatory appearance. Satellite ganglions may also ulcerate and form fistulas.

In addition to the cutaneous-lymphatic form, there is also a fixed cutaneous form that may have a stable appearance or consist of non-infiltrated plaques, generally on the face, neck and trunk [4,7].

Pulmonary and disseminated form

This form occurs following the inhalation of a large number of spores. It often gives rise to few symptoms, but it may manifest as a pneumopathy or bronchitis. Various types of lesion are observed on X ray: infiltrates, nodules, cavities, resembling other fungal pneumopathies or tuberculosis. Disseminated forms are observed mostly in immunocompromised patients with osteoarticular, secondary pulmonary and meningeal conditions [4,69].

Diagnosis

Microscopic examination of specimens

Pus, biopsy, lumbar puncture or respiratory specimens may be analysed. Fungal elements (small spherical or elongated ''cigar-shaped'' yeasts) are rare and mostly visible after Giemsa or Gram staining [4,29].

Histology

Although not specific, ''asteroid bodies'' and the Splendore-Hoeppli reaction are highly suggestive of this diagnosis. The yeasts have a thick wall surrounded by shiny eosinophilic elements and are most visible on histological sections [4,29] stained with haematoxylin-eosin-saffron, periodic acid-Schiff (PAS) or Gomori-Grocott reagents. Unfortunately, these elements are rare and, therefore, difficult to observe.

Culture

S. schenckii is a dimorphic fungus that adopts a filamentous or yeast form depending on temperature.

Filamentous form

On selective Sabouraud medium containing chloramphenicol and cycloheximide, at 20 to 30 °C, the colonies are initially white, darkening after a few days. They are smooth, creased, damp, glabrous or fluffy in appearance (Fig. 6). Under the microscope, lactophenol blue staining reveals the presence of fine filaments (1.5 μm in diameter) that are branched and septate and bear hyaline ovoid conidia. These conidia may be present singly or in groups, in the form of a bouquet (Fig. 6).

Yeast form

Yeast forms of *S. schenckii* can be isolated on blood agar at 37 °C. The colonies are smooth, white, damp and creamy. Under the microscope, the yeast cells are ovoid to globular and resemble the forms observed in specimens; they are 2.5—5.0 × 3.5—6.5 μm in size [4,69,88].

Morphological diagnosis is completed by identification through molecular analyses of clinical specimens and colonies.

Nowadays, identification is also facilitated by the use of MALDI-TOF mass spectrometry in medical laboratories. Different species have specific protein profiles, making it possible to achieve formal identification to species level in a matter of minutes. Nevertheless, although a few reference spectra are available in certain commercial databases, there are still too few and spectra for a larger number of strains are required.

Immunological diagnosis

This diagnosis is based on tests for the sporotrichin antigen from mycelia (28 °C) or yeast forms (37 °C) of *S. schenckii*. This test is based on the intradermal injection of 0.1 mL of fluid containing sporotrichin antibody into the arm. A positive result is defined as an intradermic reaction with an induration of at least 8 mm in diameter associated with erythema 48 hours later [89,90].

Other methods based on the detection of antibodies in the serum of an infected patient have also been described. They involve an immunoenzymatic technique based on serodiagnosis by immunoblotting of the exoantigens produced by *S. schenckii* in the mycelial phase. ELISA is also used for

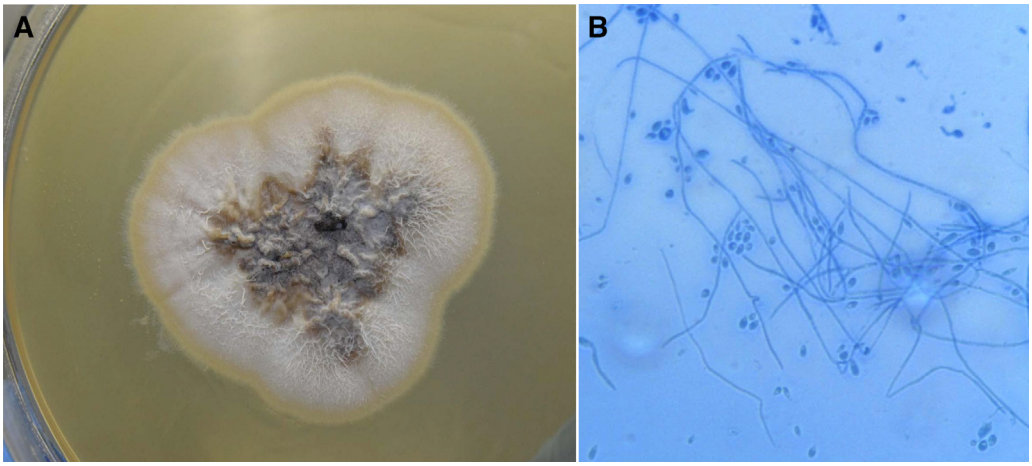

Figure 6 Macroscopic appearance of a cultured colony of *Sporothrix schenckii* after 24 days of incubation at 30 °C on Sabouraud-chloramphenicol medium (A). The colony is smooth, damp, white at the edges and brownish towards the centre. Photomicrograph (× 400) obtained after lactophenol blue staining of cultured colonies of *Sporothrix schenckii* (B). Note the fine, branched filaments surrounded by ovoid spores.

detection of the response to a peptide-rhamnomannan complex from the cellular membranes of the yeast form of *S. schenckii*. Immunodiffusion and immunoelectrophoresis methods can also be used [91,92].

Immunological diagnosis methods are widely used in epidemiological studies. However, diverse antigens are used, potentially leading to differences in the results obtained. Differences in specificity and sensitivity and cross-reactions are also observed [4].

Treatment

Potassium iodide

Potassium iodide is the treatment most widely used in countries with limited resources. It is cheap and very well tolerated [93]. Potassium iodide is administered orally, mixed with a liquid, at an initial dose of 0.5 g/day, gradually increased to 4 to 6 g/day [11]. It modulates the immune system, but its mechanism of action remains unclear. In the usual cutaneous-lymphatic forms, the disease is cured after two to three months. The treatment must then be stopped gradually, with a progressive decrease in dose. The principal secondary effects are uncommon: gastric intolerance, oedema, rash and erythema [7]. By contrast, treatment is ineffective in cases of extracutaneous SPT [88].

Itraconazole

Since the introduction of azole compounds in the 1990s, itraconazole has been the drug of choice recommended and used for the treatment of all forms of SPT [94]. Its efficacy and tolerability, even during long-term treatment, have been demonstrated in many studies [95—97]. A cure rate of 94.6% for a group of 645 patients treated with a dose of 50—400 mg/day was reported. Of the 610 patients cured, 547 were treated with a dose of 100 mg/day, 59 were treated with a dose of 200—400 mg/day and four children were treated with a dose of 50 mg/day [29,95].

Posaconazole and terbinafine

Studies of the activity of terbinafine and posaconazole against *S. schenckii* in vitro have given encouraging results [98,99]. Terbinafine at a dose of 250 to 1000 mg/day was found to be effective in human cases of cutaneous SPT [91,100]. In vitro, posaconazole is among the most active of the compounds tested and could constitute an alternative treatment. However, clinical trials will be necessary before this molecule can be considered as a treatment option [99].

In vitro tests with fluconazole, voriconazole, flucytosine and micafungin used against various strains of *S. schenckii* have shown minimal inhibitory concentrations to be high, suggesting that these antifungal drugs are not very effective against this fungus. There is currently no recommendation for the use of these antifungal drugs [101,102].

Thermotherapy

Potassium iodide and itraconazole are contraindicated in pregnant women. In such cases, thermotherapy is used. This treatment is based on the application of a heat source (a pouch of hot water or a source of infrared radiation) at 42 to 43 °C on the lesions, one to three times per day, for a total duration of 40 to 60 minutes today until the lesions resolve [69]. The heat acts on the cells of *S. schenckii* taken up by phagocytes. The efficacy of this treatment has not yet been evaluated. This treatment can be combined with the use of antifungal drugs [7].

The treatment currently used in Madagascar is 200 mg itraconazole per day for a total of six months. A very good response to treatment is observed, from the second month onwards.

Conclusion

CBM and SPT are chronic fungal infections occurring predominantly in the hot and humid zones of tropical and subtropical countries. In Madagascar, these two mycoses

are frequently encountered but remain neglected and untreated due to underdiagnosis. New active molecules, such as oral posaconazole and isavuconazole, are opening up new possibilities for the management of these diseases. Access to these treatments in countries with limited sources remains a major issue for international organizations in the domain of health. The upcoming recognition, by the WHO, of CBM as a neglected tropical disease may facilitate the implementation of national programmes. Prevention is based on knowledge of the sources of contamination. The distribution of the causal fungi in the environment has not been studied in Madagascar. Caution is required in the identification of possible sources of contamination, and molecular techniques should be used to prevent confusion with saprophytic species.

Since 2012, the Charles Mérieux Infectiology Centre (CMIC) of Antananarivo, the dermatology department of Joseph Raseta Befelatanana University Hospital and Grenoble-Alpes University have been conducting a collaborative study to update the prevalences of these two mycoses and to describe the distribution of the causal agents in the environment in Madagascar.

Disclosure of interest

The authors declare that they have no competing interest.

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