Mycetoma: a unique neglected tropical disease

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Mycetoma can be caused by bacteria (actinomycetoma) or fungi (eumycetoma) and typically affects poor communities in remote areas. It is an infection of subcutaneous tissues resulting in mass and sinus formation and a discharge that contains grains. The lesion is usually on the foot but all parts of the body can be affected. The causative microorganisms probably enter the body by a thorn prick or other lesions of the skin. Mycetoma has a worldwide distribution but is restricted to specific climate zones. Microbiological diagnosis and characterisation of the exact organism causing mycetoma is difficult; no reliable serological test exists but molecular techniques to identify relevant antigens have shown promise. Actinomycetoma is treated with courses of antibiotics, which usually include co-trimoxazole and amikacin. Eumycetoma has no acceptable treatment at present; antifungals such as ketoconazole and itraconazole have been used but are unable to eradicate the fungus, need to be given for long periods, and are expensive. Amputations and recurrences in patients with eumycetoma are common.

Introduction

Mycetoma is a mutilating, chronic, granulomatous infection of the subcutaneous tissue, which will ultimately affect deep structures and bone. Although the global disease burden is not known, mycetoma is endemic in many countries in the tropics and subtropics although most cases are reported from Sudan, Mexico, and India. It can be caused by either bacteria (actinomycetoma) or fungi (eumycetoma). In more than 80% of all cases, the foot and legs are affected.

Epidemiology

Mycetoma is a major health problem in many tropical and subtropical areas. Most cases occur in the mycetoma belt between latitudes 15° south and 30° north (figure 1).[2–13] Most cases have been reported in retrospective studies from Sudan and Mexico. In Sudan, which seems to be the most endemic country for mycetoma in the world, more than 7000 patients are receiving treatment at the Mycetoma Research Centre in Khartoum, of whom 70% are infected with the fungus *Madurella mycetomatis*. In an endemic area of White Nile State, Sudan, a field study[14] in 2010 reported a prevalence of 14·5 per 1000 population.

In a retrospective study[12] from Mexico, 3933 cases were recorded in 54 years (mean 73 per year). Of these, 97% were actinomycetoma (65% caused by *Nocardia brasiliensis* and 8% by *Actinomadura madurae*) and 3·5% were eumycetoma.[12]

The actual endemic area stretches beyond the mycetoma belt.[1,15–25] Overall most cases occur in arid and hot climates, which have a short period of heavy rainfall with milder temperatures. Actinomycetoma is more prevalent in drier areas, whereas eumycetoma is more common in sites with more rainfall. At present, more than 56 different microorganisms (bacteria and fungi) are suggested to be causative agents of mycetoma. Some of these microorganisms have been found in the soil[26–30] or the gut and casts of earthworms reared in clay loam soil,[31] suggesting that the primary niche of these microorganisms is the soil. The organism can enter the human body via a thorn prick, a wood splinter, or a stone cut.[32] Endemic areas have a savannah type of vegetation and acacia trees armed with thorns are common. In a 2014 study,[33] it was reported that the natural habitat of mycetoma causative microorganisms, at least in Sudan, is similar to that of acacia trees. The disease is not transmitted from person to person, although relatives of an individual with mycetoma living in the same locality have an increased likelihood of developing mycetoma; shared environmental factors and genetic or immunological predisposition might have a role in increasing susceptibility to the disease. Mycetoma has no known vector or animal reservoir. Mycetoma has been described in animals that have obtained the infection naturally (cats, cows, dogs, dolphins, goats, hamsters, horses, and parrots)[34–39] or via experimental induction (goats, guineapigs, hamsters, mice, monkeys, and larvae of the greater wax moth).[40–45] This diversity suggests a broad range of potential hosts. Although in dogs, goats, and horses, the causative microorganisms for mycetoma are often similar to those in human beings, in other animals the agents are usually different, albeit with similar pathological effects.[40,46]

Mycetoma affects all age groups, but it occurs most commonly in young men aged between 20 and 40 years.[8,12,47–50] In low-income and middle-income countries and endemic settings, this group generally represents the most productive and highest earning members of society. No occupation is exempt but herdsmen and farmers are most affected.[48,49]

Mycetoma is thought to be uncommon in children (3·0–4·5% of all cases in endemic settings).[12,47,51] The clinical presentation and diagnostic findings (radiology, cytology, and ultrasound) in children are similar to adults; however, amputation rates are lower, probably because of the shorter duration of disease and earlier reporting to hospital. Children are liable to become social outcasts in cases of amputation and are at risk of dropping out of school.[51]

The predominance of mycetoma in men has been consistently described. Male to female ratios are in the range of 1·6–6·6:1 both in children and adults.[12,47,49,52,53] This difference might be attributable to increased exposure in men who engage in agricultural work,

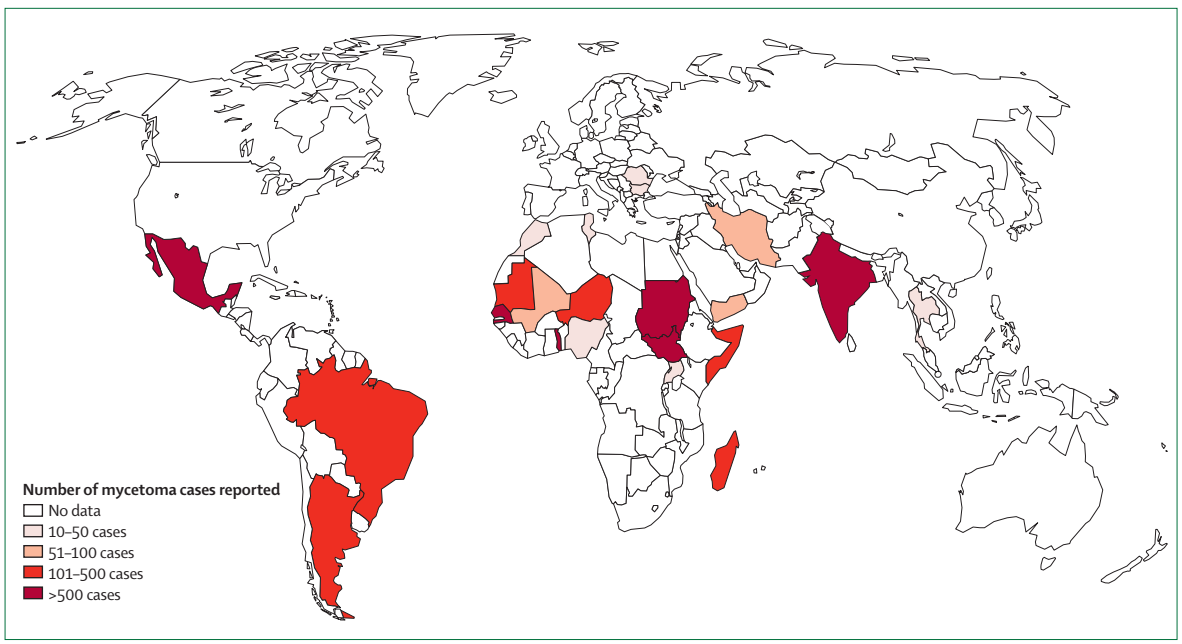

Figure 1: **Map of the global distribution of mycetoma in 2013**[1]

although in some areas this work is mainly done by women. Reporting bias should seriously be considered; in a tertiary facility in Khartoum, Sudan, the male to female ratio is 4:1, whereas at the primary care level in White Nile State, Sudan, the reported male to female ratio was more balanced (1·6:1).[54] Lastly, hormonal influences might have a role in this ratio.[55,56]

Pathogenesis

Why some individuals develop mycetoma while others do not is unclear at present. On the basis of serological surveys in endemic areas, researchers believe that most individuals have antibodies against the causative microorganisms but only a few develop disease. This variability in host response is due to the interplay between host and pathogen.[56] Patients who develop mycetoma seem to be deficient in their cell-mediated immunity.[57]

Host factors

Three types of tissue reactions are recognised in the pathology of eumycetoma—they can coexist and resemble those found in tuberculosis.[58] In type 1 reactions, the grains are surrounded by neutrophils that sometimes invade the grain and cause its fragmentation. Outside the zone of neutrophils is a zone of granulation tissue containing macrophages, lymphocytes, and plasma cells. Mononuclear cells become more numerous towards the periphery where fibrous tissue is found. Arterioles are hypertrophied and nerve cells show oedema. Hypertrophy and hyperplasia might also occur in the sweat glands.

In type 2 reactions, tissue differs from type 1 in that neutrophils have disappeared and macrophages and multinucleated cells are seen that engulf grain material. In type 3 reactions, a well organised epithelioid granuloma with Langhans' giant cells is seen. In the centre, remnant fungal material can be found; otherwise type 3 reactions are similar to type 1 and 2. Notably, the inflammatory immune response does not eliminate the grains; the giant cells that contain viable hyphae are thought to drive the formation of new grains.

In regional lymph nodes, grains and neutrophils might be evident while normal architecture is preserved. In more advanced cases, fibrosis replaces lymphoid tissue. Plasma cells with Russell's bodies are also present.

Innate immune responses are a prominent factor in mycetoma. In actinomycetoma, caused by *N brasiliensis*, neutrophils and macrophages close to *N brasiliensis* have an increased and persistent toll-like receptor 2 (TLR2) expression whereas early TLR4 expression disappears in later stages.[59]

T-cell responses seem to be important in the development of mycetoma. Th2-like responses (interleukin 10 and interleukin 4) were found in primary lesions and in draining lymph nodes in *Streptomyces somaliensis* infection and after stimulation of peripheral blood mononuclear cells by *M mycetomatis* antigens.[60–63] Macrophages stimulated with live conidia of *Pseudallescheria boydii* also induced a Th2 response, whereas hyphae induced a Th1 response.[64] Experimental infection by *N brasiliensis* in BALB/c mice causes a lesion to develop after 30 days with sinuses, microabscesses, and granules caused by expression of interleukin 10, whereas fatal dissemination occurs in homozygotic *rnu* athymic Lewis rats and similarly in nude mice infected with *Nocardia asteroides*.[65,66] Grains of *M mycetomatis* were produced in the peritoneum

of *nu nu* athymic mice after 3 weeks.[67] T-cell lymphocytes from previously immunised animals killed *N asteroides* in new infections.[66,68] The role of a pre-existing Th2 environment caused by co-infection with schistosomiasis in promoting the development of mycetoma has been suggested since patients with mycetoma were significantly more positive for schistosomiasis antibodies than healthy endemic controls.[69]

Th1 responses are found in the acute phase of infection and in healthy endemic controls.[69,70]

Humoral antibodies also have a role in pathogenesis; in immunocompetent BALB/c mice, IgM antibodies induced specific protection in experimental *N brasiliensis* infection.[71] This immune response was restricted to IgM since IgG did not show a protective effect. The disappearance of IgM antibodies and the appearance of IgG are postulated to account for the slow onset and the delay in development in experimental actinomycetoma.

The course of infection can be affected by hormonal status: in mice infected with *N brasiliensis*, treatment with testosterone and progesterone caused larger plantar lesions, whereas mice given oestradiol had smaller plantar lesions.[72] These findings seem to contrast with those in male patients with *M mycetomatis* mycetoma, since higher oestradiol blood concentrations were evident in male patients by comparison with healthy controls.[55,56]

The role of immunomodulation in drug treatment is not clear; in a mouse model of *N brasiliensis*, mycetoma treatment with diethylcarbamazine, an antifilarial compound with immunomodulatory effects, halted the progression of the infection and prevented the development of mycetoma, whereas this process was not seen for ivermectin, which also has immunomodulatory effects.[73]

Genetic association studies in healthy Sudanese controls and patients with mycetoma showed that significant differences in HLA types exist between patients and controls and in the allele distributions of genes associated with the immune response and sex hormone synthesis.[60,74,75]

Factors relating to the pathogen

In human beings, *N brasiliensis* is mostly identified in patients who are immunocompetent, whereas other *Nocardia* spp, such as *N farcinica, N nova,* and *N cyriacigeorgica*, mostly affect patients who are immunosuppressed. Irrespective of the causative microorganism, the host tissue reaction on mycetoma causative microorganisms is usually similar.[56]

N brasiliensis is resistant to some α–defensins, human neutrophil peptides 1–3, human β-defensin-3, and cathelicidin LL-37, which might enable it to survive the first-line innate immune response by phagocytes.[76] Several immunomodulators, proteolytic agents, and antimicrobials are produced by *N brasiliensis*.[77] Brasilicardin A was reported to possess immunosuppressant activity, easing implantation and progression of the infection.[77] However, this compound was not found in all strains tested and could be the product of a novel species.[78,79] *N brasiliensis* produces proteases that could assist local spread of the microorganism, which can be partly counteracted in BALB/c mice by active immunisation against these proteases.[56,77,80,81]

M mycetomatis is the most common cause of eumycetoma. It produces melanin pigments that protect microorganisms against ultraviolet radiation and destruction by alveolar macrophages, enzymatic lysis, and oxidants, and might protect against antifungal drugs. After adding melanin pigments, the minimal inhibitory concentrations increased for ketoconazole by 32 times and for itraconazole by 64 times; however, an increase in minimal inhibitory concentration due to melanin pigments has not been reported for voriconazole.[82]

Although in some studies little genetic diversity was seen between *M mycetomatis* strains from Sudan, the use of amplified fragment length polymorphism technology allowed the strains to be differentiated into two large clusters and one small cluster. The fact that mainly strains originating from large lesions were noted in one of the clusters suggests that differential virulence of the strain could have a role in the clinical presentation.[83]

Clinical presentation

M mycetomatis is the most common eumycetoma causative microorganism, whereas *N brasiliensis, A madurae, Actinomadura pelletieri*, and *S somaliensis* are the common causative organisms of actinomycetoma. The clinical presentation of actinomycetoma and eumycetoma is virtually identical, irrespective of the causative organism; however, actinomycetoma is more aggressive and destructive, and invades bone earlier than eumycetoma (table 1). The time between initial infection and consultation can vary from 3 months to 50 years. Patients might delay seeking care because of an absence of health education, poor health facilities, slow painless progression of lesions, and fear of amputation. In a study[14] published in 2014 from an endemic village in Sudan, the villagers' knowledge on mycetoma was poor in 96% of those surveyed and only 49% used satisfactory or good practices in the management of mycetoma. These findings suggest the need for health education to improve awareness in the affected communities.[14]

The triad of a painless firm subcutaneous mass, multiple sinus formation, and a purulent or seropurulent discharge that contains grains is pathognomonic of mycetoma. The outcome of infection is determined by the organism, the site of infection, and host factors that include immune responses and hormonal status. Infections start as a small subcutaneous nodule (presumably at the site of a thorn prick or other breach of the skin) that later spreads to other areas of the skin and deep structures, forming sinuses. The skin shows a somewhat tender but otherwise painless wooden induration. Eventually, the solid mass causes tissue destruction, deformity, and loss of function. Infrequently, this mass is cystic in nature.[85]

	Eumycetoma	Actinomycetoma
Epidemiology (most commonly found in)	Africa, India	Latin America
Age group	20–40 years	40–50 years
Occurs in children	Yes	Yes
Part of body affected	Feet (80%), hands (6%), other parts of arms and legs (10%), other (4%)	More extrapedal (chest, abdomen, head)
Clinical course	Less aggressive (than actinomycetoma)	Aggressive
Fistulae	Few	Many
Sinuses	Proliferative, protuberant	Flat, depressed
Bone involvement	Late	Early
Bone cavities	Large	Small
Lymphatic spread	Occasional	Frequent
Dilated veins proximal to lesion	Common	Less common (than in eumycetoma)
Macroscopic pathology	Well defined, with capsule	Ill defined, no capsule
Microscopic pathology		
Special stain	Periodic acid–Schiff	Gram, Ziehl–Neelsen
Haematoxylin and eosin stain	Fibrosis (stains strongly); filaments 2–6 μm	Fibrosis (stains weakly); filaments 0·5–1·0 μm
Ultrasound	Hyperechogenic	Less echogenic (than eumycetoma)
Treatment	Drugs and surgical	Drugs
Special characteristics	Amorphous matrix (cement) present in infections with *Madurella mycetomatis*, *Trematosphaeria grisea*, *Falciformispora senegalensis*, *Falciformispora tompkinsii*, and *Exophiala jeanselmei* but not with other eumycotic microorganisms. This amorphous matrix can be either found throughout the grain (*M mycetomatis*) or in the peripheral region only (*M mycetomatis*, *T grisea*, *F senegalensis*, *F tompkinsii*, *E jeanselmei*). *M mycetomatis* infection produces black grains that can be filamentous or vesicular, with a brown granular cement inside of the grains. *Falciformispora senegalensis* (also known as *Leptoshaeria senegalensis*) and *T grisea* (also known as *Madurella grisea*) infection produces black grains with a non-pigmented centre and no amorphous matrix, whereas in the periphery the grains are dark-coloured with brown amorphous matrix.[84]	*Nocardia* spp infections produce oval or reniform grains (80–130 μm), are Gram-positive, and partly Ziehl-Neelsen stain positive, with clubs on the periphery. *Actinomadura pelletieri* infection produces red or purplish grains with a broken dish appearance and no peripheral clubs; the colonies are multilobulated, reddish, and have fractures. *Streptomyces somaliensis* infections produce small to medium sized grains (200–800 μm), which are hard, do not have peripheral clubs, and frequently show clefts due to microtome cutting. *Actinomadura madurae* infections produce large grains about 1–5 mm in diameter, which are multilobulated, soft, and have pseudoclubs in their periphery; their colonies stains with a band located just below the pseudoclub that is more basophillic than in the colony centre.[48,66]

Table 1: **Comparison of eumycetoma and actinomycetoma**

Generally, mycetoma involves those parts of the body that come into contact with soil. The foot and hand are affected in 84% of cases, followed by other parts of the leg or arm (10%). The perineum, the abdomen, the chest, the head and neck, and the oral cavity are involved less often (each <1%). In Mexico, the back is the second most common location because rural labourers carry wood and logs on their backs; direct spread to the vertebral bodies and the spinal cord can occur with subsequent paraplegia.[48] In lesions of the skull, local invasion can cause destruction of bone and brain damage with subsequent neurological impairment (figures 2, 3, appendix).

Other typical local features of mycetoma include increased sweating in the skin overlying the lesion that corresponds with the hyperplasia and hypertrophy of sweat glands found in biopsies.[32] The local temperature is raised by increased blood flow caused by the inflammatory process. The increased blood flow to the lesion was confirmed by angiography, which showed dilated and tortuous terminal arterial branches; vascular blush and dilated veins are found proximal to the lesion and are more common in eumycetoma.[86]

Nerves and tendons are rarely affected until late in the disease; although the lesion can sometimes be tender, pain is not a feature. The clinical appearance can underestimate the extent of the lesion since undetected subcutaneous tracts can extend beyond the limits of the mass. Bone invasion can result in cavities filled with grains that contain cement-like material produced by the fungus, and fibrous tissue. Fibrous tissue provides stability and therefore pathological fractures are uncommon. Invasion of the skull by a mycetoma results in purely osteosclerotic lesions with dense bone formation.[49]

See **Online** for appendix

In mycetoma, spread of the infection occurs locally and through the lymphatics, resembling sporotrichosis.[61] Metastatic lesions can thus occur at various lymph node stations, which might become suppurative. These lymph node lesions are more common in actinomycetoma than in eumycetoma, especially in cases with repeat surgery because of recurrence. Haematological spread has also been described. *M mycetomatis* was reported in an intact blood vessel and in cases of spinal mycetoma; these lesions occurred

Figure 2: **Eumycetoma caused by *Madurella mycetomatis* with sinuses draining black grains and pus**
Picture originates from Sudan.

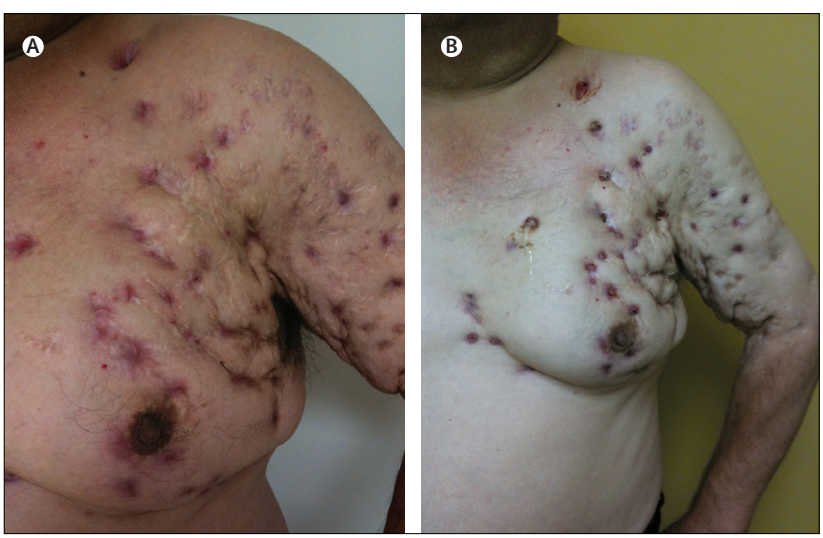

Figure 3: **Actinomycetoma caused by *Nocardia brasiliensis***
Massive infection of the chest wall, before (A) and after (B) treatment. Picture originates from Mexico.

without involvement of the skin or surrounding tissues, suggesting haematogenous seeding.[87]

Secondary bacterial infection can occur in mycetoma. In a study[88] in Sudan, 65% of patients with mycetoma had concomitant bacterial infection; of these 56% were attributable to *Staphylococcus aureus*, 34% to *Streptococcus pyogenes*, and 10% to *Proteus mirabilis*. This coexisting second deep-seated infection could contribute to the poor response to various antifungal and antibacterial drugs; elimination of these concomitant infections has been shown to improve outcome and was safe.[89]

Differential diagnosis

In endemic areas, any subcutaneous mass might be thought of as mycetoma until proven otherwise. The differential diagnosis of mycetoma is influenced by the clinical presentation, the causative microorganism, and the prevalence of infectious diseases that can mimic mycetoma.

In Sudan, where eumycetoma is common, the differential diagnosis of early lesions includes foreign body granuloma, Kaposi's sarcoma, fibroma, neurofibroma, malignant melanoma, and fibrolipoma. Large lesions that do not show sinuses should be differentiated from osteosarcoma, rhabdomyosarcoma, sporotrichosis, yaws, atypical mycobacterial infection, and tuberculosis. Primary osseous mycetoma without subcutaneous involvement should be differentiated from osteogenic sarcoma, osteomyelitis, bone cysts, and syphilitic osteitis.

Mexico has the highest prevalence of actinomycetoma. The differential diagnoses for actinomycetoma include tuberculosis, osteomyelitis, malignancies (including metastases), sporotrichosis, botryomycosis, phaeohyphomycosis, paracoccidioidomycosis, and lobomycosis.[48]

Diagnosis

Diagnosis is often made only clinically in endemic areas because of the scarcity of facilities. Ultrasound and fine needle aspiration are the minimum requirements to confirm the diagnosis and in clinical practice will diagnose most cases.

Imaging

Ultrasound is the preferred imaging technique and should be available in most district hospitals in endemic areas. Ultrasound clearly discriminates mycetoma from other subcutaneous masses. The presence of grains can be shown by sharp hyper-reflective echoes, probably caused by the grain's cement substance. In eumycetoma, cavities can be seen with or without acoustic enhancement, whereas in actinomycetoma, the grains are less distinct because of their smaller size, individual embedding, or absence of cement, and can be found at the bottom of cavities.[90] Ultrasound can be used to accurately define the extent of the lesion, which can be useful for planning surgical procedures (appendix).[90]

Radiography is useful and might be the only procedure available in peripheral hospitals. In a retrospective study of 516 patients diagnosed with mycetoma, only 3% had a normal radiograph of the affected limb. The most common abnormalities were soft tissue swelling (93%), bone sclerosis (56%), and bone invasion (46%). Other abnormalities included bone cavities (32%) and osteoporosis (19%; appendix).[91]

Helical CT is superior to plain CT scanning because it allows three-dimensional reconstruction and more precise information on the extent of organ involvement. Helical CT also allows vascular involvement to be visualised.[92]

MRI is useful for establishing the extent of the lesion and the invasion of structures. It has greater sensitivity than radiographs, ultrasound, or CT, and can also show the dot-in-circle sign that is thought to be indicative of the presence of fungal grains.[93] An MRI grading system (Mycetoma Skin, Muscle and Bone grading System) was proposed in 2012 (appendix).[94]

Identification of the organism

Identification of the causal microorganism is important to guide treatment. Grains can be obtained by extraction with a cotton swab from the sinuses or by fine needle aspiration. Deep-seated grains are preferred over those

extruded through sinuses because the latter are often not viable and are contaminated. A first indication is obtained by careful examination by eye: the size, shape, colour, and consistency of the grain aid in identification of the organism; however, it has to be isolated for its definitive identification. In direct examination, grains are mounted on a slide and crushed under a cover glass.[95] The size of the filaments, septation, morphological characteristics, and pigment formation are used to differentiate between actinomycetoma and eumycetoma (table 1).[95] In actinomycetoma, fine filaments are seen and can be stained with Gram stain. In eumycetoma, the filaments stain with periodic acid–Schiff. Grains are plated on appropriate culture media and incubated for at least 4 weeks. Identification on the basis of colony morphology can be difficult because of the large range of possible colony types and because many species that can cause mycetoma resemble each other. An absence of conidiation can further complicate identification (figure 4).

Histopathology obtained by deep biopsy has been used as the sole identification method in many centres.[96] Species can be pre-identified with haematoxylin and eosin stain.[97,98] However, many species remain practically impossible to distinguish at the species level because of their similar appearance.[95,99] In causative micro-organisms of eumycetoma, differentiation between *Scedosporium boydii, Acremonium* spp, and *Fusarium* spp, and between *Exophiala jeanselmei, Falciformispora senegalensis, Falciformispora tompkinsii, Medicopsis romeroi,* and *Trematosphaeria grisea* is also difficult.[95,99,100] Furthermore, other fungi such as *M mycetomatis* can present multiple grain types in histological slides, which complicates identification further.[32]

A stepwise approach, from the collection of specimens to the isolation and identification of established causative microorganisms of actinomycetoma, has been described by Goodfellow.[101]

Molecular identification

Chemotaxonomic methods, although effective in distinguishing between genera of actinomycetes, are laborious and time-consuming and are being complemented and replaced by molecular systematic procedures[102]—notably by 16S rRNA gene sequencing studies.[103,104] Other molecular methods championed for this purpose include PCR coupled with restriction acidonuclease analyses of PCR products,[105] PCR-randomly amplified polymorphic DNA fingerprinting, and Curie-point pyrolysis mass spectrometry.[106] Such studies provide accurate classification, especially for strains previously misclassified. However, these procedures are not available in mycetoma endemic areas.

For eumycetoma, various molecular techniques have been developed using the internal transcribed spacer as a target. To identify all fungal mycetoma causative micro-organisms, the internal transcribed spacer regions are usually amplified with pan-fungal primers and sequenced.[107] Identification is based on a comparison of the resulting sequence with sequences already present in GenBank. Using this approach, many studies have found that several causative microorganisms for eumycetoma are not differentiated at the species level, as exemplified by the identification of three new *Madurella* spp (*Madurella fahalii, Madurella tropicana*, and *Madurella pseudomycetomatis*) and *Pleurostomophora ochracea*.[108,109] For *M mycetomatis*, Ahmed and colleagues[107] developed a species-specific PCR. This PCR-restriction fragment length polymorphism analysis showed strict homogeneity between *M mycetomatis* isolates[107] and could be used to identify the causative microorganism not only from clinical material but also from soil and thorn samples.[110,111]

In 2014, a novel approach has been taken to identify mycetoma causative microorganisms, by use of the isothermal rolling circle amplification technique. With this technique, *M mycetomatis, M tropicana, M pseudomycetomatis, M fahalii, T grisea, F senegalensis, F tompkinsii,* and *M romeroi* can be easily detected within 6 h.[112]

Molecular typing of the causative microorganisms can also be done. Various methods have been used for typing of *M mycetomatis*, including restriction endonuclease analyses,[105,113] random amplified polymorphic DNA,[83,106] and amplification fragment length polymorphism.[114] Although results with random amplified polymorphic DNA are variable,[83,115] restriction endonuclease analyses[115] and amplified fragment length polymorphism[114] were able to differentiate *M mycetomatis* isolates from different countries or even within a country. Specific amplified

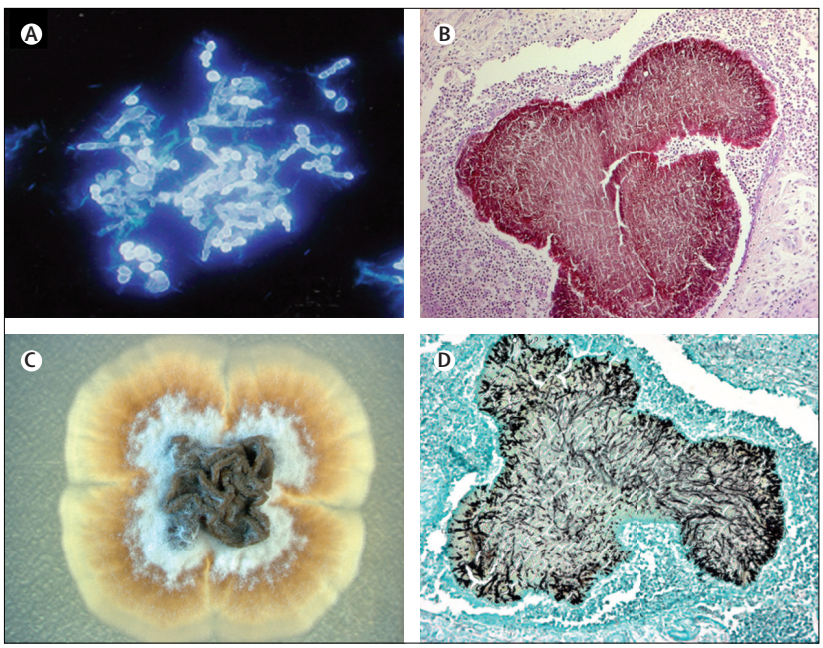

Figure 4: Madurella mycetomatis
(A) Hyphae stained with calcofluor stain. (B) Tissue section stained with haematoxylin and eosin; the fungal grain is surrounded by inflammatory cells. (C) Culture on Sabouraud agar. (D) Tissue section stained with Grocott; the fungal hyphae (black) are inside the fungal grain.

fragment length polymorphism types were associated with the origin of the strain or the size of the lesion.[114] All these tests are expensive, not available in endemic areas, and are inappropriate for use in the field.

Serology

At present, no useful serological test exists that can reliably diagnose mycetoma. Several serological assays have been used, including immunoblots,[116] indirect haemagglutination assays,[117] immunodiffusion,[118] counter-immunoelectrophoresis,[118] and ELISA.[119]

Salinas-Carmona and colleagues[119] described the use of ELISA for the serological diagnosis of *N brasiliensis*, the most common microorganism causing actinomycetoma in Mexico. This study revealed a higher incidence of antibodies in patients with active disease without cross-reactions with *Mycobacterium leprae* and *Mycobacterium tuberculosis*. This technique has been useful in cases where identification of the causative microorganism in culture was not possible.[119]

For microorganisms that cause eumycetoma, serological assays have been developed only for *M mycetomatis* and *P boydii*. Immunodiffusion, indirect haemagglutination assays, and counterimmunoelectrophoresis use crude antigens that are not standardised and are not sensitive and specific enough.[118,120] An ELISA based on pure antigens of *M mycetomatis*—including the recombinant produced translationally controlled tumour protein (TCTP) and the luminex assays based on TCTP—fructose-bisphosphate aldolase, and pyruvate kinase also had insufficient specificity and could not differentiate between patients and healthy endemic controls.[121,122]

	Range (mg/mL)	MIC_{90} (mg/mL)
Amphotericine B[82,123]	<0·01 to 4	2
Terbinafine[124]	1 to >16	8
Azoles		
Fluconazole[82]	0·25 to >128	16
Ketoconazole[82]	<0·01 to 1	0·125
Itraconazole[82,123]	<0·01 to 0·5	0·06
Isavuconazole[125]	<0·01 to 0·125	0·06
Posaconazole[124]	<0·03 to 0·125	0·06
Voriconazole[82]	<0·01 to 1	0·125
Ravuconazole[126]	≤0·002 to 0·031	0·25
5-flucytosine[82]	>128	>128
Echinocandins		
Anidulofungin[127]	0·5 to >128	>128
Caspofungin[127]	16 to >128	128
Micafungin[127]	8 to >128	>128
Artemisinin[128]	0·03 to >16	>16
Tea tree oil[128]	0·008 to 0·25	0·25

MIC_{90}=minimum inhibitory concentration for 90% inhibition.

Table 2: **In-vitro sensitivity for antifungal drugs and potential new drugs to treat mycetoma caused by *Madurella mycetomatis***

In the absence of reliable point-of-care tests, at present, in endemic areas, a fine needle aspiration for cytology and cell blocks are recommended to identify the causative organism to start with and if needed, to proceed with taking a deep surgical biopsy sample for histological and mycological identification. Further molecular typing can be done later in specialised laboratories.

Treatment

Treatment depends on causative organism

Early mycetoma with a small lesion is amenable to treatment and has good prognosis. Generally, actinomycetoma is responsive to medical treatment although it requires long-term treatment and is expensive. The treatment of eumycetoma is disappointing, requiring long periods of antifungals combined with surgical management. Recurrence is common and can be related to non-compliance and an absence of response or inadequate response to antifungals and surgery. Of all antifungal drugs available at present, only drugs belonging to the azole class have sufficient in-vitro sensitivity against *M mycetomatis* (table 2). At present, drugs are entirely restricted to the azole group; no alternative drugs are in preclinical development.

Eumycetoma

Ketoconazole at doses of 400–800 mg/day for 9–12 months has been the mainstay of treatment for decades (table 3). However, in 2013 its use was restricted by the US Food and Drug Administration because of potentially fatal liver injury, drug interactions, and adrenal gland problems.[140] For the same reasons the European Medicines Agency recommends suspension of marketing authorisations. Therefore, at present, itraconazole is recommended for use. Itraconazole is not curative but it reduces the lesion size, with formation of fibrosis enabling less mutilating surgery; the fungus itself can still be isolated from the surgical material.[132] Terbinafine has been used in small numbers of patients with limited efficacy.[133] Voriconazole and posaconazole have been assessed in a very limited number of patients with promising results (table 3); however, despite good in-vitro activity, long duration of treatment seems to be needed. Isavuconazole and fosravuconazole were reported to have excellent in-vitro activity.[125,126]

In-vitro studies have shown that the melanin pigments produced by *M mycetomatis* increased the minimum inhibitory concentrations for itraconazole by 32 times and ketoconazole by 64 times, whereas the minimum inhibitory concentrations for amphotericin B, fluconazole, and voriconazole were not affected.[82] In view of these results, voriconazole could be effective as a monotherapy or for reducing the duration of treatment. The effect of melanin on increasing minimum inhibitory concentrations for posaconazole has not been studied. Amphotericin B has poor activity in vitro and is toxic after prolonged routine use. Limited

	Organism (number of cases)	Dose	Outcome	Country
Ketoconazole[129]	*Madurella mycetomatis* (13 [8 from Sudan and 5 from Saudi Arabia])	200–400 mg once a day; median treatment duration is 13 months (range 3–36 months)	5 cured and 4 improved	Sudan and Saudi Arabia
Ketoconazole[130]	*M mycetomatis* (50)	200 mg twice a day for 3–36 months	36 (72%) were cured or had notable improvement; 10 (20%) had some improvement; 4 (8%) had no response or deteriorated	Sudan
Ketoconazole[131]	*M mycetomatis* (4), other (4)	400 mg once a day for 8–24 months	6 cured, no recurrence after 3 months (2 years follow-up); 2 improved	India
Itraconazole[132]	*M mycetomatis* (13)	200 mg twice a day for 3 months, then 200 mg once for 9 months	1 cured; 12 improved and cured after surgery; 1 recurrence	Sudan
Terbinafine[133]	*M mycetomatis* (10), *Falciformispora senegalensis* (3), other (3), not known (7)	500 mg twice a day for 24–48 weeks	4 cured; 11 improved	Senegal
Voriconazole[134]	*Scedosporium apiospermium* (1)	400 mg once a day for 18 months	Cured	Côte d'Ivoire
Voriconazole[135]	*S apiospermium* (1)	Dose not specified; 6 months duration	Cured	India
Voriconazole[135]	*Trematosphaeria grisea* (1)	Dose not specified; 6 months duration	Little change	India
Voriconazole[136]	*M mycetomatis* (1)	200 mg twice dailyfor 3 months, then 300 mg twice daily for 13 months	Cured	Mali
Voriconazole[137]	*Madurella* spp (1)	200 mg twice daily for 12 months	Cured	Senegal
Voriconazole[138]	*S apiospermum* (1)	200 mg twice a day; unknown duration	Cured (after 3 years follow-up)	Brazil
Posaconazole[139]	*M mycetomatis* (2), *T grisea* (3), *S apiospermum* (1)*	400 mg twice daily for a maximum of 34 months	Initially 5 were cured and 1 had no improvement; 2 were successfully retreated after interval of >10 months	Brazil
Liposomal amphotericin B[130]	*T grisea* (2), *Fusarium* spp (1)	Total dose 3·4 g and 2·8 g (*T grisea* cases), and 4·2 g (*Fusarium* spp case); maximum daily dose is 3 mg/kg	All showed temporary improvement but relapsed within 6 months	Not specified

*All refractory cases.

Table 3: **Treatment of eumycetoma in endemic cases and immunocompetent patients**

	Organism (number of cases)	Dose	Outcome	Country
Co-trimoxazole[145]	*Actinomadura pelletieri* (60), *Actinomadura madurae* (25), *Streptomyces somaliensis* (5)	1600/320 mg orally, once a day for 1 year	75 (83%) cured	Senegal
Amikacin and co-trimoxazole[66]	*Nocardia brasiliensis* (48), *A madurae* (4), *Nocardia asteroides* (1), *Nocardia* spp (2), *S somaliensis* (1)	Amikacin: 15 mg/kg intramuscularly or intravenously; co-trimoxazole: 960 mg orally for 3–5 weeks (1 cycle)	19 cured after 1 cycle, 15 cured after 2 cycles, 18* cured after 3 cycles, 4 cured after 4 cycles,	Mexico
Imipenem alone or with amikacin[146]	*Nocardia* spp (5)	Imipenem: 500 mg intramuscular injection, three times a day; amikacin: 15 mg/kg intramuscularly or intravenously, once a day over a 3 week cycle, repeated every month until cure	3 cured; 2 improved	Mexico
Gentamicin and co-trimoxazole initial phase, then doxycycline and co-trimoxazole maintenance phase[147]	8 confirmed, not specified; 8 clinically diagnosed	Gentamicin (initial phase): 80 mg/kg intramuscularly, twice a day for 4 weeks; co-trimoxazole (initial phase): 320/1600 mg twice a day for 4 weeks; doxycycline (maintenance phase): 100 mg orally, twice a day; co-trimoxazole: 320/1600 mg orally, twice a day for 5–6 months	16 improved after initial phase; 9 of 12 in maintenance phase cured; during follow-up, 6 of 7 remained cured; 1 had a recurrence	India
Rifampicin and co-trimoxazole[148]	Not specified (1)	Rifampicin: 600 mg orally, once a day; co-trimoxazole: 960 mg orally, twice a day	Cured after 10 months	India
Rifampicin and co-trimoxazole[144]	Not specified (5)	Rifampicin: 4·3 mg/kg orally, once a day; co-trimoxazole: 960 mg orally, twice a day, mean 9 months	1 cured; 4 greatly improved; no failure	Sudan
Co-amoxiclav[149]	*N brasiliensis* (19), *N asteroides* (1), *Nocardia otitidiscaviarum* (1)	875/125 mg orally, twice a day, mean 9·6 months	15 cured; 2 improved; 4 did not improve	Mexico

*One patient had a recurrence.

Table 4: **Treatment of actinomycetoma in endemic cases and immunocompetent patients**

data on liposomal amphotericin B exists. Echinocandins have no in-vitro activity against *M mycetomatis* (table 2).[127]

An effective and safe antifungal with a short duration of treatment is urgently needed for eumycetoma. Frustrated with the high cost and treatment duration, patients in Sudan revert to herbal medicine leading to side-effects and further delay in treatment.[141] Whether monotherapy is clinically useful or if the combination of

Panel: Major knowledge gaps in mycetoma

Epidemiology
- Accurate data on incidence, prevalence, and geographical distribution

Diagnosis
- Sensitive and specific serological tests or DNA-based tests such as PCR that can be used at point-of-care
- Identification of biomarkers that can be used to monitor therapeutic outcome
- Generation of species-specific oligonucleotide probes to accurately identify the causative microorganisms directly from clinical material (grains) by use of whole-genome sequencing studies

Mode of transmission
- Identification of the natural habitat of the causative organisms (soil, dung, other)
- Identification of the primary reservoir and alternative or intermediate hosts
- Risk factors for transmission; practicality and efficacy of wearing shoes in prevention
- Presence of subclinical infection and risk factors for progression
- Possible role for co-infections

Therapy
- Explore correlation between in-vitro sensitivity of azoles (ketoconazole, itraconazole, isavuconazole, voriconazole, posaconazole, and ravuconazole, but not fluconazole) with in-vivo clinical response
- Explore other compounds that are not azoles

Search strategy and selection criteria

We searched the electronic database PubMed using the terms "mycetoma" or "madura foot" or "actinomycetoma" or "eumycetoma" or "*Nocardia*" or "*Madurella*" for manuscripts published between July 1, 1898, and June 30, 2015. We included manuscripts in English, French, Spanish, and Portuguese. From the references of each article, we explored further references as appropriate. Both human and animal infections were included.

two antifungals would provide better efficacy with less toxic effects and reduced treatment duration still needs to be resolved. Concomitant treatment for bacterial superinfection should be considered and the importance of co-infection, such as with schistosomiasis, deserves further study.[69,88]

At present, the recommended treatment for eumycetoma is itraconazole 200–400 mg/day for 6–9 months followed by wide local excision if the lesion is not fully cured by the drug. Postoperatively itraconazole is continued until the patient is clinically, radiologically, ultrasonically, and cytologically cured. A patient is deemed clinically cured when the skin becomes normal, the mass disappears, the sinuses heal, and the organisms are eliminated from the tissue. Radiological examination is essential for follow-up of patients on medical treatment and to assure cure. It usually shows reappearance of normal bone pattern and the disappearance of the soft-tissue mass. The absence of grains in a fine needle aspiration with a type 3 tissue reaction and the disappearance of the grains and cavities on ultrasonography are reliable indicators for cure.[58]

Actinomycetoma

More information is available on the treatment of actinomycetoma than eumycetoma, although well controlled comparative studies are scarce.[66,67,142,143] Early studies by Mahgoub[144] showed that actinomycetoma caused by *A pelletieri*, *A madurae*, and *N brasiliensis* responded well to antibiotic treatment, whereas infection with *S somaliensis* was more difficult to treat. Combinations of co-trimoxazole with either dapsone or streptomycin were significantly more effective than co-trimoxazole alone for mycetoma caused by all four species. At present, the first-line treatment is thought to be 48 mg/kg per day of co-trimoxazole (trimethoprim and sulfamethoxazole in a ratio of 1:5) in cycles for 5 weeks and amikacin 15 mg/kg per day in a divided dose every 12 h for 3 weeks. The 2 week interval of amikacin in the 5 week cycle is used for renal and audiometric monitoring. Of 56 patients treated with this regimen, all but one were cured after a maximum of four cycles.[66] In case of resistance or allergy to co-trimoxazole or amikacin, co-amoxiclav can be used as an alternative to co-trimoxazole and netilmicin to amikacin (table 4). Co-amoxiclav can also be used alone during pregnancy; however, acquired resistance might develop, and it is generally not effective against *A madurae*.[66] Amikacin combined with a carbapenem, such as imipenem or meropenem, could also be used in refractory cases. Surgery is rarely needed.[66] Determination of the minimum inhibitory concentrations of the carbapenems in each patient is recommended because of resistant strains and the cost of these drugs.

Conclusions

Mycetoma is a unique neglected tropical disease, affecting very poor people in remote communities. Serious knowledge gaps exist, particularly in epidemiology, transmission, and clinical management (panel). The disease burden needs to be defined. Existing diagnostic methods are inadequate and cannot be used at point of care. The treatment of mycetoma, particularly for eumycetoma, is far from optimum, and is characterised by a low cure rate despite long treatment duration, with many patients dropping out only to present later with recurrences that can be even more difficult to treat than the initial presentation. The available antifungal drugs are not effective, toxic, expensive, and not available in endemic areas. For actinomycetoma, more simple treatment regimens are needed than exist at present with uninterrupted administration of drugs to avoid development of resistance.

Preventive measures such as promotion of footwear have not been explored.

To bridge the knowledge gaps, improved global awareness on mycetoma is needed. Commitment of local

health authorities is essential to improve the health system, to raise awareness in the community, to provide public education, and to train local health workers. Early case detection and treatment will reduce the high morbidity and improve outcome.

Contributors

EEZ initiated and wrote the first draft, after which all authors contributed equally.

Declaration of interests

We declare no competing interests.

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