Between latitude 30° North and 15° South

Pubmed: Mycetoma reported from 50 countries
Incidences, prevalence and mapping of mycetoma

van de Sande, 2013

- Madurella mycetomatis
- Streptomyces somaliensis
- Actinomadura madurae
- Actinomadura pelletieri
- Nocardia brasiliensis
Mycetoma

Definition: **chronic infection of subcutaneous tissues**

---

**Actinomycetoma** (bacteria)
- Nocardia brasiliensis
- Nocardia asteroides
- Streptomyces somaliensis
- Actinomadura madurae
- Actinomadura pelletieri

**Eumycetoma** (fungi)
- Madurella mycetomatis
- Falciformispora senegalensis
- Trematosphaeria grisea
- Scedosporium apiospermum
- Acremonium spp.
Mycetoma

Definition: chronic infection of subcutaneous tissues

Actinomycetoma (bacteria)

Eumycetoma (fungi)

Similar presentation

More aggressive
Treatment with antibacterial agents

Slow progression
Treatment with antifungals
The mode of transmission

van de Sande, 2013
Epidemiology
White Nile State
Sudan

- Transmission:
  - Thorn prick, (micro-)trauma
  - Most cases on feet, but not exclusively
  - People often barefooted

- Reservoir: unknown
  - Plants, soil, animal dung, other?
Mapping the Potential Risk of Mycetoma Infection in Sudan and South Sudan Using Ecological Niche Modeling

Abdallah M. Samy,1,2* Wendy W. J. van de Sande,3 Ahmed Hassan Fahal,4 and A. Townsend Peterson1

* Correspondence: samy@cam.ac.uk
The mode of transmission

- Natural habitat → soil?

- Culture:
  - *M. mycetomatis* was cultured from Indian soil in 1968
  - Thirumalacher et al. Hindustan Antibiot Bull. 1968

- PCR:
  - 17/74 soil samples positive for *M. mycetomatis*
  - 1/22 thorn samples positive for *M. mycetomatis*
Van Hellemont, PLoSNTD 2013
Spread along the lymphatics
Mycetoma diagnosis

- Clinical
- Microbiological
- Serological
- Radiological
Mycetoma diagnosis

• Clinical
  • subcutaneous mass
    – foreign body, ganglion, fibroma, neurofibroma, rhabdomyosarcoma, Kaposi’s sarcoma, sporotrichosis, lobomycosis, etc
  • sinuses
    – tuberculosis
    – malignancy
  • discharge – grains colour
### Mycetoma diagnosis – clinical: grains colour

<table>
<thead>
<tr>
<th>Colour</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>Madurella mycetomatis, Trematospheria grisea, Exophiala jeanselmei, Medicopsis romeroi, Falciformispora senegalensis, Curvularia lunata</td>
</tr>
<tr>
<td>Red</td>
<td>Actinomyces pelletieri</td>
</tr>
<tr>
<td>Yellow-brown</td>
<td>Nocardia brasiliensis, Nocardia otiditiscaviarum, Actinomadura madurae, Streptomyces somaliensis</td>
</tr>
<tr>
<td>Yellow</td>
<td>Pleurostomophora ochracea</td>
</tr>
</tbody>
</table>
Grains are easily demonstrated either by spontaneous discharge or by FNA.
Microbiological diagnosis – grains

A. Black grains – *M. mycetomatis*
B. Yellow grains – *S. somaliensis*
C. *M. mycetomatis* – in formalin
D. *M. mycetomatis* – grain in tissue
E. *S. somaliensis* – grain in tissue

van de Sande, PLoS NTD 2014
Microbiological diagnosis – culture: macroscopic, microscopic

A. *M. mycetomatis*
B. *M. mycetomatis*, microscopic calcofluor white stain
C. *Nocardia brasiliensis*
D. *N. brasiliensis*, microscopic

Disadvantages
- Time consuming
- Contamination
- Experience needed

Misclassifications occur!

van de Sande, PLoS NTD 2014
Serodiagnosis

- **Actinomycetoma**
- **Eumycetoma**
  - Crude antigens
  - Recombinant antigens
  - No distinction between patients and endemic controls

Photograph showing a counterimmunoelectrophoresis test with positive bands.
Serology

- **Antigens**
  - β-D-glucan
    - Fungus cell wall
    - Not specific
    - Commercially available
Molecular diagnosis

• **Standard:** Amplification of ITS region and sequencing
  – Expensive equipment needed:
    • Thermocycler
    • Sequencer
  – Time consuming
Lane 1 contain a 100 bp DNA ladder.

Lanes 2 to 4: PCR products for three samples which were negative for *Madurella mycetomatis*.

Lane 5 to 8 :PCR products for four samples which were positive for *Madurella mycetomatis*.

Lane 10 : positive control

Lane 11 : negative control
Molecular diagnosis

- **Isothermal amplification** to identify black grain causative agents of mycetoma
  - Loop-mediated isothermal amplification (LAMP)
  - Recombinase polymerase amplification (RPA)

- **Genome sequencing**
  - Smit 2016: *M. mycetomatis* strain
  - Lucio Vera-Cabrera 2014: *Actinomadura* strain
Ahmed AA. PLoS NTD 2017

Diagram:
- FNAC
  - FNAC Positive
  - FNAC Negative
    - Biopsy Surgical
      - Grains Culture
      - Histopathological Examination
      - Molecular Techniques
### Key characteristics of eu- and actino-mycetoma

<table>
<thead>
<tr>
<th></th>
<th>Eumycetoma</th>
<th>Actinomycetoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causative agent</td>
<td>Fungi</td>
<td>bacteria</td>
</tr>
<tr>
<td>Main endemic area</td>
<td>Africa</td>
<td>Middle- and South America</td>
</tr>
<tr>
<td>Treatment</td>
<td>Antifungal + surgery</td>
<td>antibiotics</td>
</tr>
<tr>
<td>Current regimen</td>
<td>Ketoconazole</td>
<td>amikacin (IV) + cotrim (PO)</td>
</tr>
</tbody>
</table>

**Drug Safety Communications**

**FDA Drug Safety Communication:** FDA limits usage of Nizoral (ketoconazole) oral tablets due to potentially fatal liver injury and risk of drug interactions and adrenal gland problems.

- or Itraconazole
- 12 Months + mass removal

**Cure Rate**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37% → 25.9%</td>
<td>&gt; 90% (in Mexico)</td>
</tr>
<tr>
<td>Organism (number of cases)</td>
<td>Dose</td>
<td>Outcome</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>Ketoconazole138 <em>Madurella mycetomatis</em> (13 [8 from Sudan and 5 from Saudi Arabia])</td>
<td>200-400 mg once a day; median treatment duration is 13 months (range 3-36 months)</td>
<td>5 cured and 4 improved</td>
</tr>
<tr>
<td>Ketoconazole330 <em>M mycetomatis</em> (50)</td>
<td>200 mg twice a day for 3-36 months</td>
<td>36 (72%) were cured or had notable improvement; 10 (20%) had some improvement; 4 (8%) had no response or deteriorated</td>
</tr>
<tr>
<td>Ketoconazole331 <em>M mycetomatis</em> (4), other (4)</td>
<td>400 mg once a day for 8-24 months</td>
<td>6 cured, no recurrence after 3 months (2 years follow-up); 2 improved</td>
</tr>
<tr>
<td>Itraconazole332 <em>M mycetomatis</em> (13)</td>
<td>200 mg twice a day for 3 months, then 200 mg once for 9 months; cured; 12 improved and cured after 2 years follow-up; 1 recurrence</td>
<td>Sudan</td>
</tr>
<tr>
<td>Terbinafine333 <em>M mycetomatis</em> (10), <em>Falciformispora senegalensis</em> (3), other (3), not known (7)</td>
<td>500 mg twice a day for 24-48 months</td>
<td>11 improved</td>
</tr>
<tr>
<td>Voriconazole334 <em>Scedosporium apiospermum</em> (1)</td>
<td>400 mg twice a day; 6 months duration</td>
<td>Cured</td>
</tr>
<tr>
<td>Voriconazole335 <em>S apiospermum</em> (1)</td>
<td></td>
<td>Cured</td>
</tr>
<tr>
<td>Voriconazole336 <em>Trematosphaeria grisea</em> (2), <em>S grisea</em> (9), <em>S apiospermum</em> (1)</td>
<td></td>
<td>Little change</td>
</tr>
<tr>
<td>Voriconazole336 <em>M mycetomatis</em> (1)</td>
<td>300 mg twice daily for 3 months, then 300 mg twice daily for 13 months</td>
<td>Cured</td>
</tr>
<tr>
<td>Voriconazole207 <em>Madurella spp</em> (1)</td>
<td>200 mg twice daily for 12 months</td>
<td>Cured</td>
</tr>
<tr>
<td>Voriconazole338 <em>S apiospermum</em> (1)</td>
<td>200 mg twice a day; unknown duration</td>
<td>Cured (after 3 years follow-up)</td>
</tr>
<tr>
<td>Posaconazole339 <em>M mycetomatis</em> (2), <em>T grisea</em> (3), <em>S apiospermum</em> (1)*</td>
<td>400 mg twice daily for a maximum of 34 months</td>
<td>Initially 5 were cured and 1 had no improvement; 2 were successfully retreated after interval of &gt;10 months</td>
</tr>
<tr>
<td>Liposomal amphotericin B330 <em>T grisea</em> (2), <em>Fusarium spp</em> (1)</td>
<td>Total dose 3.4 g and 2.8 g (<em>T grisea</em> cases), and 4.2 g (<em>Fusarium spp</em> case); maximum daily dose is 3 mg/kg</td>
<td>All showed temporary improvement but relapsed within 6 months</td>
</tr>
</tbody>
</table>

*All refractory cases.

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

• Host
  – spectrum
  – size, duration
  – immune response
  – co-infection; super-infection

• Fungus
  – type
  – melanin
Blood supply and vasculature in advanced mycetoma

- Arteriography (n=9)
  - Normal femoral, popliteal, tibial arteries
  - Circulation brisk and excessive (in contrast to tumours)
  - Early venous filling, increased veins
  - No AV shunts

- Doppler (n=60)
  - No difference in BP between affected and normal limb (ankle-brachial index)

- Histology (n=30)
  - Endarteritis obliterans in 7% (low in granulomatous disease)
  - Arteritis 7%
  - No thrombosis, fibrinoid necrosis, ischaemia or invasion of vessels by microorganisms

Post-operative mycetoma surgical specimen (H&E x 40)

- colonies of black grains
- surrounded by thick fibrous capsule
- intensive fibrosis

**Sudan: study with itraconazole: n=13**

- Treatment for 12 months
- 1 cured
- 9 partial response

Fahal et al. TRSTMH 2010
HE stain

Rich in lipid, protein, Zn, Cu, Ca and melanin

Calcium stain

Melanin stain
MIC shift with melanin from *M. mycetomatis* (grey) and melanin from *C. neoformans* (white)
From: vd Sande, Microb Infect 2007)
## Antifungal Susceptibility

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (range) µg/ml</th>
<th>Antifungal</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (range) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole</td>
<td>0.125 (&lt;0.01-1)</td>
<td>Amphotericin B</td>
<td>2 (&lt;0.01-4)</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.06 (&lt;0.01-0.5)</td>
<td>Terbinafin</td>
<td>8 (1-&gt;16)</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.06 (&lt;0.03-0.125)</td>
<td>5-flucytosine</td>
<td>&gt;128 (&lt;128)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>16 (0.25-&gt;128)</td>
<td>Caspofungin</td>
<td>128 (16-&gt;128)</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.125 (&lt;0.01-1)</td>
<td>Anidulafungin</td>
<td>&gt;128 (0.5-&gt;128)</td>
</tr>
<tr>
<td>Isavuconazole</td>
<td>0.06 (&lt;0.01-0.125)</td>
<td>Micafungin</td>
<td>&gt;128 (8-&gt;128)</td>
</tr>
<tr>
<td>Ravaconazole</td>
<td>0.004 (&lt;0.002-0.03)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Study design: «drop the loser»

- **Randomisation**
  - fos - 300
  - fos - 200
  - itra

- **3 months**

- **Interim analysis**

- **Surgical removal of encapsulated lesion**
  - fos - 200 or 300
  - itra

- **3 months**

- **12 – 13 months**
  - 6 months
## Alternative drugs

<table>
<thead>
<tr>
<th>MIC</th>
<th>range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisinin</td>
<td>0.03-16</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Tea tree oil</td>
<td>0.008-0.25</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>&lt;0.002-0.06</td>
<td>0.008</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*vd Sande, JAC 2007*

**NSAIDs**

- as adjuvant therapy to antifungals
  - influence inflammation – cytokine profile; Th2 ➔ Th1

*Dupont, LID 2016*
63% have secondary bacterial infection of those: 56% *S. aureus*; 34% *S. pyogenes*, 10% *P. mirabilis*

*Ahmed et al. J Clin Microbiol 1998*
Conclusion and Priorities

- No alternatives for azoles in eumycetoma
- Global burden
- Collection of strains
  - *in vitro* sensitivity
- Itraconazole generic formulations
- (Open access) drug discovery including medicinal plants