Black mycelial fungal infections – Asian perspectives

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Black Mycelial Fungal Infection
Asian Perspectives

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Dematiaceous fungi

• a heterogeneous group of fungi characterized by their production of melanin pigments in their hyphae & conidia.
• widely found in nature e.g. soil, wood, decomposing plant matter, & polluted water
• Rarely cause infection but has wide range of diseases especially in immuno-compromised patients (transplant recipients, HIV infected)

Brandt & Warnock J Chemother 2003; 15 - Suppl. 2 (36-47)
https://drfungus.org/knowledge-base/fonsecaea-species/
Clinical manifestations

Superficial infection: dermatomycosis & onychomycosis

Subcutaneous infection: chromoblastomycosis, sporotrichosis, etc

Systemic infection: visceral organ incl. cerebral infection

Brandt & Warnock J Chemother 2003; 15 - Suppl. 2 (36-47)

Dermatomycosis

Tinea nigra

- Hortaea werneckii
- Chronic cutaneous infection, in the palm (►) & sole (◄),
- Central & South America, Africa, & Asia

Diagnosis:

Treatment:
- Terbinafine
- Iodine & salicylic acid alcohol sol.

The fungus & clinical occurrence

Kuo et al, Dermato Sinica 1993; 11:47-53
Dermatomycosis

**Black piedra**
- *Piedraia hortae*
- Infect hair (scalp)
- humid tropical regions: South & Central America, Africa, **Southeast Asia**, Africa, & the South Pacific islands.
- Nature: soil?, still unknown
- young adults, men > women
- Spread: common use of combs, hairbrushes or other hair utensils

**The fungus**


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Subcutaneous infection
Sporotrichosis

- Subcutaneous infection
- Caused by dimorphic fungus S. schenckii
- Saprophyte in soil, sphagnum moss, decaying vegetation & hay
- Asia: mostly reported from India, Japan & Australia

Chakrabarti & Slavin Med Mycol 2011, 49, 337–44

Chromoblastomycosis

The causes & other characteristics
- The causes dematiceaous fungi e.g. *F. pedrosoi* species complex
- Microscopy of clinical specimen: round, thick-walled, brown cells, or sclerotic bodies
- Prevalent in Asia & Latin America

Clinical presentation
- common: erythematous & verrucous plaques, nodules
- Cicatricial & tumorous (less)

Yang et al. Medical Mycology, 2017, 0, 1–11
Clinical presentation of chromoblastomycosis

Figure 1. Clinical manifestations of chromoblastomycosis (CBM) cases. (A–C) Uncommon locations of CBM, including the ear lobe (A), cheek (B), and areola (C). (D–E) Well-defined papuloplaque-type lesion, easily misdiagnosed as psoriasis or Bowen’s disease. (F–H) The verrucous type was the most common presentation in our study, with a verruciform surface and black dots. (I–K) Cicatricial type of lesion with an stippled border and central clearance. (L) Targetoid type lesions are very rare and present with three zones, namely the central nodule, clearance, and rimmed elevated border.

Chromoblastomycoses: epidemiology

<table>
<thead>
<tr>
<th>country</th>
<th>Cause (s)</th>
<th>demography</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td><em>F. nubica</em></td>
<td></td>
<td>Yanagihara et al. JDA 2015 (CR)</td>
</tr>
<tr>
<td>China</td>
<td><em>C. carrionii, F. monophora, F. pedrosoi &amp; Phialophora verucosa</em></td>
<td>10-79 y.o., male&gt;female (5.21:1) &amp; farmers,</td>
<td>Lu et al Mycopathologia DOI 10.1007/s11046-012-9586-z</td>
</tr>
<tr>
<td>Indonesia</td>
<td>?</td>
<td>Adult, male&gt;female, 3 cases</td>
<td>Yahya et al. J Gen Pro DVI. 2016;1(2):36-43</td>
</tr>
<tr>
<td>Taiwan</td>
<td><em>F. monophora &amp; F. nubica</em></td>
<td>male&gt;female (2.75:1); 29-90 y.o.</td>
<td>Yang et al. Med Mycol. 2017; 0, 1–11</td>
</tr>
</tbody>
</table>
A subcutaneous infection caused by dematiaceous fungi
Systemic infection

Clinical manifestations & its causes

Cerebral & systemic infection
- Cerebral
  - Cladophialophora bantiana
  - Ramichloridium mackenziei
  - Exophiala dermatitidis
  - Lomentospora prolificans
- other systemic infection
  - Endocarditis
  - Osteomyelitis

Source in nature

Soil & decaying vegetation in tropical countries

Brandt & Warnock J Chemother 2003; 15 - Suppl. 2 (36-47)
Kelly et al. BMC Infectious Diseases. 2016; 16:36
Steinbach et al. JCM 2003; 41: 3981–5
Lang et al. BMC Infectious Diseases (2018) 18:255
C. bantiana
A known neurotropic fungus

C. bantiana brain infection

- is a melanized hyphomycetous, highly virulent fungus & the most common cause of brain infection
- High mortality due many factors e.g.
  - Delay of diagnosis
  - (No) standardized treatment

Chakrabarty et al. Med Mycol, 2016, 54, 111–9
1. India (39 - 32.5%),
2. United States (29 - 24.2%)
3. Brazil (each 5 - 4.2%)

Source in nature
• soil saprophyte
• can be found in living or dead plants
• individuals who work with soil

Inhalation: sinus or pulmonary infection

Hematogenous dissemination to the brain

Skin trauma causes skin inoculation

Risk factors

Immunocompetent
59.2% patients

Immunosuppressed
40.8% patients


Report from Asian Countries

- Other Asian countries: Japan, Vietnam, Thailand & Indonesia
- Immunocompetent patients with no clear risk factors except from Indonesia (IVDU)
- **Patients in Asia are generally young**
  - **Japan**: women aged around 20s with cerebral cladosporiosis
  - **Indonesia**: A brain infection in a IVDU young male


Indonesian case

- A 31 year old male, drug user (IVDU) complained of head ache, left side weakness & slurred in speech, which worsening from time to time
- By his family he was sent abroad & was diagnosed as brain abscess, caused by *Aspergillus*
- He was treated with voriconazole without any improvement & send back to Jakarta
- Since his condition deteriorate, MRI was conducted (irregular area enhancement at T1-T2) & LP was done & no organisms were isolated, later on surgical excision was conducted
- Material from brain abscess was sent to our lab. & *C. bantiana* was isolated & identified based on phenotypic method.
Indonesian case

- a possible risk factor was identified.
- Possibly the fungus enter the body via skin trauma by the injection of syringe (an IVDU)
- The wound caused by the usage of non-sterile needle is the entrance of the fungus into the body & subsequently disseminated to the brain


Diagnosis

Based on clinical presentation
Based on laboratory result: collection of clinical specimens
Imaging
Clinical manifestation - atypical

- Headache (56.1%)
- Hemiparesis (54.2%)
- Seizures (31.8%)
- Altered sensorium (30.8%)
- Fever (22.4%)
- Vomiting (18.7%)
- Aphasia/dysarthria (14.9%)
- Visual disturbance (11.2%)


Diagnosis

- Clinical symptoms – atypical – misdiagnosis
  - The mean duration of symptoms before diagnosis was ± 115 days
- Laboratory: hard to collect samples due to location in the brain
- Once the fungus is isolated:
  - Phenotypic identification – morphology
  - Histopathology
  - Nutritional studies
  - Genetic analysis based on sequencing of rDNA

Morphology: macroscopy

- Colony is black, velvety (RT & 37°C)
- Long, non-fragile chains of conidia

Histopathological stains

H&E stain of melanized, moniliform hyphal elements of *C. bantiana* from a brain abscess.

Giemsa stain of abscess specimens

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Revankar & Sutton. CMRev. 2010;23(4):884-928;
Kantarcioğlu et al, Med Mycol 2016; 5: 579-604
Genetic analysis & Nutritional physiology

- **Primary region:**
  Internal transcribed spacer (ITS) region using ITS1 and ITS4 primer
- **Alternative region:**
  - Large subunit (LSU) region
  - Small subunit (SSU) region
- Maximum temperature for growth 42°C.
- *Methyl-a-D-glucoside, meso-erythritol & ethanol* assimilated
- No or poor growth with L-arabinitol
- Good or weak growth with ribitol & galactitol


Treatment

- Surgical excision + Antifungal

- Overall mortality rate was 65%
- Higher in immunosuppressed patients (77.1%) than immunocompetent hosts (56.3%)

Antifungal susceptibility testing

<table>
<thead>
<tr>
<th>S. no</th>
<th>AmB</th>
<th>Flu</th>
<th>Vor</th>
<th>Itra</th>
<th>Posa</th>
<th>Casp</th>
<th>Ani</th>
<th>Mica</th>
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<td>4</td>
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<td>&gt; 64</td>
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<tr>
<td>12</td>
<td>1</td>
<td>&gt; 64</td>
<td>0.06</td>
<td>0.250</td>
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<tr>
<td>13</td>
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<td>64</td>
<td>0.250</td>
<td>0.125</td>
<td>0.03</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Adapted from: Chakrabarti et al. Med Mycol 2016;54:111–9

Antifungal

- standard guideline: ESCMID – ECMM for black fungi
- Personal experience
- Amphotericin B is the most widely used drug (70.4%), followed by flucytosine (34.6%)
- Recommendation: combination of amphotericin B and flucytosine
Lomentospora (Scedosporium) prolificans

- Rarely reported in Asia
- One case from Japan, a 68 yo woman with lung infection
- Treated with voriconazole

Infection caused by *E. rostratum*

A newly recognized neurotropic mould
Life cycle of *Exerohilum rostratum*

E. *rostratum* in nature

- A soil saprophytes
- Pathogen in plant – banana leaf & others, causes black spot disease
- Biocontrol/herbicides agent against red sprangletop (*Leptochloa chinensis*), a pest to rice plant
- 2012 an outbreak of meningitis; a new insight for neurotropic characteristic

http://www.dannyhaelewaters.com/exserohilum-rostratum-the-killing-fungus/
**Exerothilum rostratum** – neurotropism

- October 2012, US Federal official reported meningitis in 214 people, then 304 people after having injection of steroid to their epidural space for back pain
- The steroid was contaminated by *E. rostratum*
- This fungus was directly inoculated to the human tissue & induces response
- Causes severe inflammatory response marked by high concentration of leukocytes in LCS
- *E. rostratum*
  - black mould;
  - synthesize melanin - associated with virulence & resistance to both polyene- & echinocandin-type antifungal agents

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**E. rostratum**: susceptibility to Anti fungal

<table>
<thead>
<tr>
<th>Antifungal Susceptibility: <em>Exerothilum rostratum</em> (Australian National data); MIC µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmB</td>
</tr>
<tr>
<td>VORI</td>
</tr>
<tr>
<td>POSA</td>
</tr>
<tr>
<td>ITRA</td>
</tr>
</tbody>
</table>

*E. rostratum* data from 34 isolates (da Cunha et al. 2012); MIC µg/mL
- AmB range <0.03-0.125: MIC<sub>50</sub>= 0.03
- VORI range 0.03-0.25: MIC<sub>50</sub>= 0.25
- ITRA range <0.03-0.125: MIC<sub>50</sub>= 0.03
- POSA range <0.03-0.5: MIC<sub>50</sub>= 0.06

CDC suggestion is Voriconazole 6mg/Kg BW/daily

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**Bipolaris hawaiiensis**

Culture of *B. hawaiiensis* in LPCB


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**B. hawaiiensis**

- Prev. *Dreschlera hawaeiensis*
- Optimum temperature is 25-35°C, but able to grow at 37°C
- A problem in the storage of crops (post harvest disease)
- Means able to grow in human tissue & causes infection

Almaguer et al. Aerobiologia DOI 10.1007/s10453-012-9257-z
Pic. Wikimedia
**B. hawaiensis**: range of disease

- **ABPA (allergy) in pediatric** (Chowdary et al. Med Mycol. 2011;49:760-5)
- **14/17 developed endophthalmitis after intra vitreal of triamcinolone from the same lot.** (Small et al. Ophthalmology 2014; 121:952-8)
- **8 eyes of 8 patients developed endophthalmitis after intra vitreal injection of bevacizumab-triamicinolone combination.** (Sheyman et al. JAMA Ophthalmol. 2013;131(7):864-9)

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**Susceptibility of B. hawaiensis to antifungal agents**

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th><strong>B. hawaiensis</strong> (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anidulafungin</td>
<td>0.07 - 0.125</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.125 - 0.25</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>0.5 - 1</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>&lt;0.03 - 0.5</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>2 - 32</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Micafungin</td>
<td>&lt;0.015 - 0.06</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>&lt;0.03 - 0.5</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.25 - 2</td>
</tr>
</tbody>
</table>

Damage response framework (DFR)

- Black mycelial fungi rarely cause disease, but once an infection occurs there will be severe damage to the host tissue. This can be explained by DFR.
- Tissue damage in infection is not merely due to microbial activity.
- Interaction between host- micro organism determine tissue damage which associate with pathological outcome.
- The damage is the result of microbial activity & host response.
- In case of mycosis this theory applies e.g. B. hawaiensis & E. rostratum were previously known as non pathogenic to human, but direct inoculation in epidural space & steroids allows changes that facilitate infection.

My opinion

Asian perspective - conclusion

- Melanized fungi are able to cause infection in human with broad clinical spectra; from cutaneous to cerebral infection.
- Mostly found in humid tropical countries such as many regions in Asia, but report from Asia is limited.
- The humid climate in Asian countries is a fertile medium for fungal growth & the environment is an important source of infections.
- Climate change (deforestation, pollution etc.) allows fungi to adapt to high temperatures so that infections in humans are very likely to occur.
- Some fungi are clinically significant, some are unclear, but we need to be aware because their ability to adapt to the human body paves the way for infection.
- Both clinicians & laboratories must be prepared to deal with it.
Thank you