Case Reports

A rare complication of ear piercing: a case of subcutaneous phaeohyphomycosis caused by *Veronaea botryosa* in China

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We present the third case of phaeohyphomycosis caused by *Veronaea botryosa* in China and the tenth case worldwide. A 16-year-old Chinese girl developed crusted, verrucous lesions, initially on the left ear and later on the left buttock, within 2–5 months of receiving an ear piercing. Histopathological examination of biopsy specimens confirmed diagnosis of subcutaneous phaeohyphomycosis. Microscopic examination of the colonies recovered in culture from a portion of the biopsy specimen resulted in the identification of *Veronaea botryosa* based primarily on the presence of two-celled, brownish pigmented, cylindrical conidia produced sympodially from erect conidiogenous cells. The lesions significantly improved with daily oral treatment with itraconazole 400 mg and adjuvant thermotherapy for 6 months. A maintenance therapy with low dose itraconazole was prescribed in order to achieve clinical and mycological cure. A two-year follow-up didn’t reveal any recurrence of infection. Our case is the first report of *V. botryosa* infection associated with a cosmetic procedure, which suggests that skin piercing could precipitate *V. botryosa* or other dematiaceous, as well as opportunistic fungal infections.

**Keywords** *Veronaea botryosa*, Phaeohyphomycosis, subcutaneous, treatment

Introduction

Phaeohyphomycotic infections, caused by a diverse group of melanized fungi, are being increasingly reported worldwide. Melanized fungi are commonly found as saprophytes in soil, on plants and in water and are characterized by the production of dark melanin pigment in the mycelium and conidia. There are only ten reported cases of phaeohyphomycotic infections caused by *Veronaea botryosa*, primarily from Asia. In this report we present the third case of subcutaneous phaeohyphomycosis due to *V. botryosa* in China, as well as a brief review of literature on *V. botryosa* infections. Body piercing or other cosmetic procedures could potentially increase the risk of phaeohyphomycosis and such a possibility needs attention.

Case report

A 16-year-old girl from Jiangsu Province, China, complained of crusted verrucous skin lesions over her left ear and left buttock. The patient had received a cosmetic piercing in her left ear lobule, which was done following routine procedures and was unremarkable. Two months later, several 3 × 3 mm papules with exudates appeared sporadically around the piercing area on the left ear and gradually became crusted verrucous lesions which spread to almost the entire preauricular areas of the ear (Fig. 1a). Over the subsequent 5 months, an infection developed on the left buttock with similar lesions (Fig. 1b) which probably resulted from scratching. In the interim, she had seen a
local dermatologist and had been treated with topical steroids and antifungals, which did not result in improvement of the lesions. The latter had gradually enlarged and become 5 × 5-mm nodules, which in 50% of the ear had coalesced with adjacent ones to form plaques, leading to a deformity of the left ear (Fig. 1a).

Fig. 1  (a) Verrucous, crusted, nodular lesions on the left ear and extending to preauricular skin areas, and (b) a well-demarcated, erythematous, scaly lesion on the left buttock of a 16-year-old Chinese girl before treatment. Lesions on (c) the left ear, and (d) the left buttock of the patient significantly improved 1 month after treatment.
The patient was a high-school student and had no active gardening, soil or water exposure. She had no history of immune diseases or infections and her family history was unremarkable. Physical examination revealed multiple, non-tender, erythematous, crusted nodules or plaques on the left ear and left buttock. Superficial lymph nodes were non-palpable. No abnormal findings were noted in a blood test, urine test, chest X-rays, cranial CT and abdominal ultrasound. Skin biopsies from the lesions on the left ear and buttock were obtained and subjected to microscopic, histopathologic and fungal cultural examinations. The isolate recovered in culture was identified as *Veronaea botryosa* according to the key characteristics described by Ellis [1] and de Hoog et al. [2]. Antifungal therapy was initiated with oral itraconazole 400 mg daily with adjuvant therapy for 6 months. A significant clinical improvement was noticed, however, a follow-up over a longer period of time was desired to assess the long-term outcome of the treatment. In a 2-year follow-up the lesions were resolved and there was no evidence of recurrence.

**Histopathologic examination**

Sections of the biopsy tissues collected from the left ear and left buttock were stained with haematoxylin and eosin (H&E), periodic acid Schiff’s reagent (PAS), and Gomori methenamine silver (GMS) procedures. Microscopic examination of the H&E-stained tissue revealed a chronic granulomatous inflammation infiltrated with lymphocytes, histiocytes, neutrophils, and multinucleated giant cells. The epidermis was thickened, irregular, and showed pseudoeppitheliomatous hyperplasia. Septate, branched or unbranched and pale brown hyphae of various lengths were observed in the tissue (Fig. 2a). Examination of the PAS (Fig. 2b) and Gomori methenamine silver stains (Fig. 2c) also demonstrated the presence of septate hyphae.

**Mycological examination**

Portions of skin biopsies from verrucous lesions were inoculated onto potato dextrose agar plates containing chloramphenicol and cycloheximide. The culture plates were incubated at 25°C and 37°C in the dark and examined every other day. After two weeks, numerous velvety, olivaceous gray colonies were visible on all plates at both at 25°C and 37°C, but appeared to grow more rapidly at 25°C. No growth was evident at 40°C. Bacterial and mycobacterial cultures were negative. Scrapings from crusts of the lesions were examined in KOH mounts revealing numerous septate, branched, yellowish to brownish hyphae, 2.5–4.0 μm in diameter. The conidiophores were erect, straight or flexuous, occasionally branched, rarely geniculate, smooth-walled, septate, pale brown, bearing cylindric, smooth-walled, 0–2 septate (predominantly 1-septate) conidia (Fig. 2d, e). The fungal isolate hydrolyzed urea and was nitrate positive and grew on media containing cycloheximide. The fungus was identified as *Veronaea botryosa* based on its morphological characteristics [1,2]. The isolate has been deposited in the Institute of Dermatology, Chinese Academy of Medical Sciences, Peking Union Medical College, Nanjing, China under accession number CMCC(FD.28C).

**Molecular testing**

Genomic DNA was extracted using Biopsin Fungus Genomic DNA Extraction Kit (Bioer Technology, China) according to the manufacturer’s protocol. The universal fungal primer pair was used for amplification: ITS1(5’-TCCGTAGGTAACCTGCGG-3’) and ITS4(5’-TCCTCCGCTTATTGATATGC-3’). The PCR assay was performed using 0.5 μl of the test DNA sample in a total reaction volume of 25 μl. The PCR master mix consisted of 11 μl of nuclease-free water, 0.5 μl stock of each primer (10 μM), 12.5 μl of 2 × Go Taq Green Master mix (Promega Corporation). Thirty-five cycles of amplification were performed in a BioRad MyCycler thermocycler after initial denaturation of DNA at 95°C for 10 min. Each cycle consisted of a denaturation step at 94°C for 30 s, an annealing step at 55°C for 40 s, and an extension step at 72°C for 1 min, with a final extension at 72°C for 7 min following the last cycle. Following amplification, an approximately 601-bp amplicon was detected.

DNA sequencing of the PCR product was done at the Shanghai Sangon Biological Engineering Technology Service Company on ABI PRISM 3730DNA sequencer using BigDye terminator v3.1 according to the manufacturer’s protocols. The PCR product was directly sequenced using both the ITS1 primer and ITS4 primer. The resultant nucleotide sequences were aligned to produce consensus for analysis. The consensus sequence of the isolate aligned with 99% sequence similarity to multiple sequences of *V. botryosa* available in the GenBank database.

**Drug sensitivity test**

*In vitro* antifungal susceptibility testing was performed through the use of a macrobroth modification of the previously published Standard M38-A from ‘National Committee for Clinical Laboratory Standards’ reference method for broth dilution antifungal susceptibility testing of filamentous fungi [3]. *In vitro* minimal inhibitory concentrations (MICs) of several antifungals against *V. botryosa* were as follows; itraconazole (2 μg/ml), fluconazole (>64 μg/ml), terbinafine (1 μg/ml), ketoconazole (2 μg/ml), miconazole (> 4 μg/ml), bifonazole (> 4 μg/ml), nystatin (4 μg/ml), amphotericin B (1 μg/ml).
Table 1  Review of 10 cases of phaeohyphomycosis due to *Veronaea botryosa*.

<table>
<thead>
<tr>
<th>Country</th>
<th>Age (yr)</th>
<th>Time of disease progress before diagnosis</th>
<th>Gender</th>
<th>Occupation</th>
<th>Preceding event or underlying conditions</th>
<th>Site of lesions</th>
<th>Description of lesions</th>
<th>Treatment and outcome</th>
<th>Year of report</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henan Province, China</td>
<td>24</td>
<td>10 yrs</td>
<td>M</td>
<td>Farmer</td>
<td>ND</td>
<td>Back of left hand, forearm, both cheeks</td>
<td>Black verrucous nodules and cysts</td>
<td>ND</td>
<td>1990, 1991</td>
<td>7, 8</td>
</tr>
<tr>
<td>Tripoli, Libyan</td>
<td>28</td>
<td>3 yrs</td>
<td>F</td>
<td>ND</td>
<td>ND</td>
<td>Upper limb, thumb and 5th finger of right hand, nasal mucosa, and palate</td>
<td>Nodular or ulcero-nodular lesions and a flexure deformity</td>
<td>ND</td>
<td>1995</td>
<td>9</td>
</tr>
<tr>
<td>Philippines</td>
<td>37</td>
<td>3 yrs</td>
<td>M</td>
<td>Machine worker</td>
<td>ND</td>
<td>Right deltoid, left shin</td>
<td>Erythematous, pruritic papules and nodules</td>
<td>ITZ</td>
<td>1998</td>
<td></td>
</tr>
<tr>
<td>La Reunion Island, Indian Ocean</td>
<td>57</td>
<td>11 wks</td>
<td>M</td>
<td>Sugarcane farmer</td>
<td>Liver transplant and immunosuppressive therapy</td>
<td>Both feet, wrists and forearm</td>
<td>Nodules and pus formation</td>
<td>Prolonged ITZ 300mg/d. for 8 mo. Cured</td>
<td>1999</td>
<td>10</td>
</tr>
<tr>
<td>Taiwan, China</td>
<td>81</td>
<td>ND</td>
<td>M</td>
<td>Retired farmer</td>
<td>ND</td>
<td>Left dorsal foot, lymphatics of the left leg</td>
<td>Erythematous swelling plaque with pustules and sinus tracts and several fluctuating papulonodules along lymphatics</td>
<td>Partial surgical debridement and ITZ 200 mg daily; Clinical improvement and negative fungal culture</td>
<td>2003</td>
<td>11</td>
</tr>
<tr>
<td>Jiangsu Province, China</td>
<td>12</td>
<td>6 yrs</td>
<td>M</td>
<td>Student</td>
<td>Scratch on the right arm</td>
<td>Widely spread to right elbow, face, upper limbs, legs, scrotum and buttocks</td>
<td>Crusted nodules and plaques with exudates</td>
<td>Herbal treatment for &gt;1 yr without improvement. Then, providone-iodine baths,</td>
<td>2003</td>
<td>12</td>
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(Continued)
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<tr>
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<th>Year of report</th>
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<tr>
<td>Houston, Tex., US</td>
<td>62</td>
<td>3 mo</td>
<td>M</td>
<td>ND</td>
<td>Heart transplant. Antibiotics and immunosuppressive therapy</td>
<td>At site of IV line insertion on the dorsum of the right hand</td>
<td>Induration, erythema, and intense tenderness</td>
<td>local heat treatment and AMB facial injections, TERB 125 mg daily for 6 mo plus ITZ 100 mg daily for 6 mo. Lesions in some regions progressed and no improvement in others; Currently no treatment due to financial difficulties.</td>
<td>2004</td>
<td>13</td>
</tr>
<tr>
<td>Taiwan, China</td>
<td>76</td>
<td>&gt;2 yrs</td>
<td>M</td>
<td>Retired farmer</td>
<td>History of diabetes, Coronary artery disease, and Cushing’s syndrome</td>
<td>Right forearm, left upper limb, and right knee</td>
<td>Multifocal, crusted, brownish-red noduoplaques</td>
<td>Incision and drainage of the lesion area; ITZ 200 mg BID for 1 wk; VORI 200 mg BID for 10 wk. cured, lesions resolved in 1.5-yrs follow-up.</td>
<td>2006</td>
<td>14</td>
</tr>
<tr>
<td>Japanese</td>
<td>65</td>
<td>3 yrs</td>
<td>F</td>
<td>Farmer</td>
<td>ND except for history of hepatitis C.</td>
<td>Dorsum of the left wrist</td>
<td>An erythematous, slightly scaly, indurated plaque</td>
<td>ITZ 200 mg daily for 6 mo. Slowly progressed without improvement. Resistance to ITZ and AMB Topical steroids, antifungals and vitamin D3 without improvement. Surgical excision</td>
<td>2007</td>
<td>15</td>
</tr>
</tbody>
</table>
Discussion

More than 130 fungal species belonging to 70 diverse genera have been reported as causal agents of human and animal phaeohyphomycosis, among which V. botryosa is rarely recognized as a causative pathogen [4, 5]. Phaeohyphomycosis: definition and etiology. Proceedings of the Third International Conference on Mycoses Scientific Publication No. 304. Washington, DC. Pan American Health Organization No. 304. Washington, DC. Pan American Health Organization, 1975; 126 – 130. In spite of the global distribution as evidence of the recovery of V. botryosa from soil samples in Brazil [6], China [Nishimura K, Miyaji M, Taguchi H, et al. An ecological study on the biotopes of dematiaceous fungi in China. In: Current Problems of Opportunistic Fungal Infections. Proceedings of the 4th International Symposium of the Research Center for Pathogenic Fungi and Microbial Toxicoses. Chiba: Chiba University, 1989; 17 – 20], Egypt, USA, and India; plant materials from Italy; air materials in New Zealand, Australia, and alligator farms in Queensland, Australia, human infections have been few. There have been only a total of ten cases of human infections, including our case. Because of the rarity of V. botryosa as a human pathogen and the novelty of phaeohyphomycosis as a complication of the lesion, resulted in complete healing.

Abbreviations: M, male; F, female; ND, not determined; ITZ, itraconazole; VORI, voriconazole; AMB, amphotericin B; TERB, terbinafine. *Age at diagnosis.

The patient received treatment in Jinling Hospital, Nanjing, China, with oral itraconazole, 400 mg daily, with adjuvant thermotherapy for 1 yr with significant improvement as early as 1 mo. After 1-yr therapy, ITZ 100 mg continued for 6 mo. 2-yr follow-up showed no evidence of recurrence of the infection and liver function tests were normal.
ear piercing, we deem it worthwhile to report this case. It presents a new scope in pathogenesis of phaeohyphomycosis regarding predisposing factors and effective therapeutic regimen for *V. botryosa* or other dematiaceous infections.

The patient, in the present case, had a unique history of injury from a cosmetic procedure. *V. botryosa* infection was preceded by piercing of the ear lobules which may have resulted, a few months later, in a severe skin lesion. It was speculated that *V. botryosa* had gained entrance through the use of inadequately sterilized tools in the ear piercing or probably more likely, from the environment. The wound created a disintegrated skin barrier which precipitated the infection. Since skin is an anatomical barrier from external environmental pathogens, the integrity of skin plays an important role in the body’s defense. Therefore, damage of skin could predispose individuals to opportunistic fungal infection, as in the present case.

In the nine human *V. botryosa* infections reported in the literature, therapeutic regimes were quite variable, but it appears that itraconazole is the most effective oral antifungal for phaeohyphomycosis. In the present case, combined therapy of oral itraconazole and local thermotherapy proved to be highly effective. The addition of local thermotherapy could be recommended as a useful adjuvant of antifungal therapy for *V. botryosa* or other dematiaceous infections. In addition, the initial dose of itraconazole is critical, as a low dose could lead to resistance. The therapeutic strategy we applied has shown its benefit in all cases of phaeohyphomycotic infections we have thus far encountered.

The ability of *V. botryosa* to grow at 35°C, albeit slowly, potentiates invasive disease, especially in immunocompromised patients [10,13]. This first report of phaeohyphomycosis following ear piercing should alert dermatologists and warned of the potential for opportunistic fungal infections.

### Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

### References


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