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Phaeohyphomycosis caused by *Corynespora cassiicola*, a plant pathogen worldwide

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ABSTRACT

Although rare, trans-kingdom infection features an interesting infection biology concept, in which highly versatile pathogenic attributes allow successful infections in evolutionarily highly divergent species. *Corynespora cassiicola* is a phytopathogenic fungus and occasionally causes human infections. Herein, we report a phaeohyphomycosis case caused by *C. cassiicola*. Given that sporadic reports may contribute to a lack of awareness of the transmission route, clinical manifestations, and diagnostic and clinical management, we systematically reviewed the cases reported thus far. Nine patients were identified and included in the pooled analysis, 88.9% (8/9) of whom were reported after 2010. All patients were from Asian, African, and Latin American countries, among whom 77.8% (7/9) were farmers or lived in areas with active agriculture. Exposed body parts were the major affected infection area, and clinical manifestations were mainly non-specific inflammatory reactions. Although biochemical and morphological examinations confirmed the presence of fungal infection, molecular analysis was used for the final diagnosis, with 77.8% (7/9) being identified by internal transcribed spacer sequencing. Whereas voriconazole, terbinafine, and AmB, either alone or in combination, resulted in successful infection resolution in most cases (5/9; 55.5%), those suffering from invasive facial infections and *CARD9* deficiency showed poor outcomes. Our patient is the third case of invasive facial infection caused by *C. cassiicola* and was successfully treated with intravenous LAmB followed by oral voriconazole combined with topical antifungal irrigation. Molecular identification of fungus and prompt antifungal treatment is pivotal in the clinical success of patients suspected to have phaeohyphomycosis. Moreover, as evidenced by our data, itraconazole treatment is not recommended.

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Corynespora cassiicola; phaeohyphomycosis; voriconazole; terbinafine; amphotericin B

1. Introduction

Most invasive human fungal infections are caused by species belonging to *Aspergillus, Candida*, and *Cryptococcus* and are associated with significant mortality rates (Brown et al. 2012; Hong et al. 2017; Ghazanfari et al. 2021). However, advancements in clinical intervention (immunosuppression, transplantation, chemotherapeutic drugs, etc.) (Bongomin et al. 2017), the increasing number of patients identified with in-born genetic deficiencies (Lanternier et al. 2015) and sprawling human habitats to vegetative areas, where plant pathogens can employ their versatile pathogenic attributes and cause infection in humans (Jackson et al. 2019), have potentially increased the risk of obtaining infection with rare pathogens.

For instance, in rare cases, a plant pathogen named *Corynespora cassiicola* belonging to dematiaceous fungi, which can infect more than 530 plant species of 380 genera, including monocots, dicots, and ferns (Dixon et al. 2009), is known to cause an array of infections in humans (Mahgoub 1969; Huang et al. 2010; Lv

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et al. 2011; Yamada et al. 2013; Yan et al. 2016; Arango-Franco et al. 2018; Wang et al. 2018, 2019; Xie et al. 2018). There are a wide variety of dematiaceous fungi that can cause human infections, with more than 130 known species of 70 genera (Revankar and Sutton 2010), and new species are constantly being identified. First described by Ajello in 1974, phaeohyphomycosis is defined as subcutaneous and invasive infections caused by opportunistic pathogenic dematiaceous fungi, which are characterised as yeast-like cells and/or dark brown septate hyphae in the tissue (Isa-Isa et al. 2012).

Given that scarcity and sporadic cases concerning phaeohyphomycosis caused by *C. cassiicola* in humans can potentially contribute to a lack of awareness and underestimation of this fungal pathogen, we conducted a literature review to systematically assess the microbiological and clinical factors of published cases (Mahgoub 1969; Huang et al. 2010; Lv et al. 2011; Yamada et al. 2013; Yan et al. 2016; Arango-Franco et al. 2018; Wang et al. 2018, 2019; Xie et al. 2018) and our case to fill this knowledge gap and to provide a more reliable reference for the treatment of affected patients.

2. Materials and methods

2.1. General clinical information

A 17-year-old male student visited our clinic complaining of a nasal dorsum bulge with a peculiar smell for more than two months. There was no obvious incentive for the patient to have a peculiar smell in the nasal cavity two months ago, which was not taken seriously and dealt with. Afterward, it was gradually found that a local bulge appeared on the nasal root, but there were no discomfort symptoms during the period, such as fever, tenderness, runny nose, and headache. At this point, he was referred to the hospital for treatment. Sinus CT (computerised tomography) examination showed diffuse soft tissue thickening and bone resorption destruction on the bilateral nasal roots, dorsum of the nose, and anterior and upper parts of the nasal septum. Electronic nasopharyngeal endoscopy was performed, which showed neoplasms in bilateral nasal cavities, narrowing of each nasal passage, mucosal congestion of the posterior wall of the nasopharyngeal roof, and a raised and uneven surface. The patient refused surgical treatment and was given empiric antibacterial therapy, which was not effective, with progressive swelling of the posterior nasal root and extension to the dorsum of the nose. Therefore, the endoscopic nasal mass biopsy was performed under general anaesthesia, and fungal infection could not be excluded from postoperative pathology.

Subsequently, the patient was referred to our clinic, and due to the observation of numerous fungal spores and filament structures using microscopic evaluations of the nasal secretion, the patient was diagnosed with fungal infection. Therefore, he was treated with 200 mg twice daily oral itraconazole for two weeks. Although the patient felt that the peculiar smell in the nasal cavity disappeared, the nasal dorsum bulge gradually progressed. He lived in an agriculture county with no history of underlying diseases, had recurring epistaxis only one year ago, and denied a premorbid history of local trauma involving soil or plants. For further diagnosis and treatment, he was admitted to the hospital.

On admittance, she underwent an examination showing an ill-defined, hard, unpushable local bulge on the dorsum of the nose with no redness, ulceration, tenderness, or skin temperature increase, along with no localised or generalised lymphadenopathy (Figure 1). The results of routine laboratory tests revealed a normal leukocyte count 6,800/μL (55.1% granulocytes, 0.7% eosinophils), erythrocyte sedimentation rate 4 mm/h (ESR, normal value 0–15), and procalcitonin 0.024 ng/mL (PCT, normal value 0–0.5), slightly elevated C-reactive protein 13.49 mg/L (CRP, normal value 0–10), decreased CD4+/CD8+ T-cell count 1.08 (normal value 1.57–2.93), elevated serum 1,3-β-D-glucan 242.9 pg/mL (G test, normal value 0–100), and elevated serum immunoglobulin E 364 IU/mL (Ig E, normal value 0–100). No significant abnormality was found in any other comprehensive related examinations, such as tumour markers or autoimmune indicators.

With minor clinical improvement, oral itraconazole was discontinued and replaced by intravenous voriconazole 200 mg twice daily based on the results of the *in vitro* antifungal susceptibility test (please see results section). However, three days later, the treatment had to be stopped because the patient developed intolerable visual impairment. At this point, intravenous LAmB (AmB liposomal) was started, with an initial dose of 50 mg once daily, and no obvious

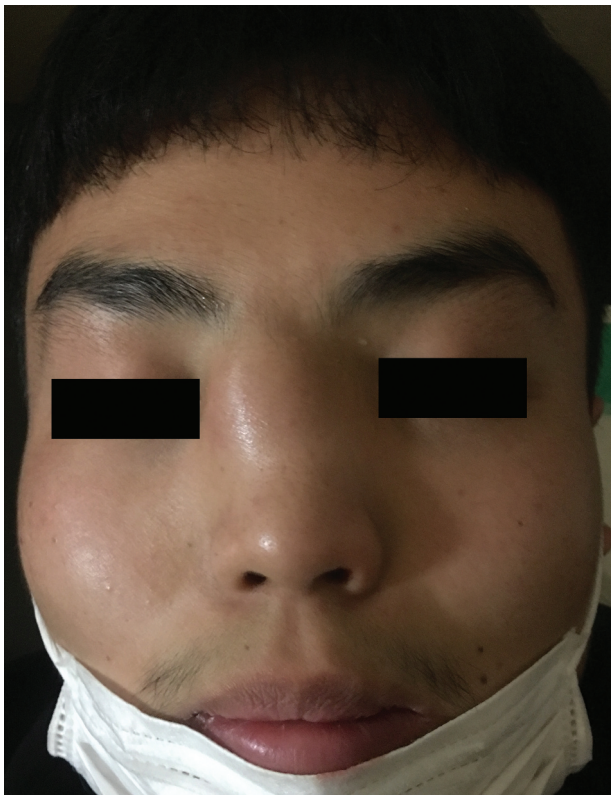

Figure 1. A local bulge on the dorsum of the nose upon admission.

side effects were noted in the patient. Then, the daily dose of intravenous LAmB was gradually escalated to a maintenance dose of 100 mg, and 20 mg LAmB was mixed with 50 mL 5% glucose solution to wash the nasal cavity once daily. One month later, nasal endoscopy showed that the mass in the nasal cavity was smaller than before, and no fungal spores or hyphae were found by microscopic examination of nasal secretions. Hence, intravenous LAmB was replaced by oral voriconazole 200 mg twice daily, with no visual impairment recurrence. Then, the patient was discharged, but oral voriconazole 200 mg twice daily was continued at the outpatient clinic. At the follow-up examination, no reactivation of the fungal infection was found, and to date, the patient is still under close follow-up observation.

2.2. Methods

To find more evidence of fungal infection, periodic acid-Schiff staining (PAS) was performed on tissue sections. The biopsy collected from the nasal cavity under nasal endoscopy was cultured on potato dextrose agar (PDA; Oxoid, UK) and incubated at 26 °C and 37 °C. After one week of incubation, the initial isolate was subcultured onto Sabouraud dextrose agar (SDA; Oxoid, UK) in small culture steel rings. One week later, a direct microscopic examination was performed after the materials were stained with lactophenol cotton blue and 10% potassium hydroxide (10% KOH).

The isolate was further identified at the species level by amplification and sequencing of the internal transcribed spacer (ITS), β-tubulin (*BT2*), and D regions, and the determined fragments were submitted to a nucleotide database (BLASTn) for nucleotide comparison with known fungal sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Then, we used the maximum likelihood method to construct a phylogenetic tree to clarify the relationship between related species groups.

Using the M38-A2 criterion (CLSI 2008) recommended by the American Clinical Laboratory Standardization Institute (CLSI), *in vitro* susceptibility of the isolate to nine common antifungal drugs was tested, including fluconazole (FZ), itraconazole (IZ), voriconazole (VOR), posaconazole (PZ), caspofungin (CAS), micafungin (MF), anidulafungin (AND), amphotericin B (AmB), and 5-fluorocytosine (5-FC).

2.3. Literature review

We systematically reviewed patients diagnosed with phaeohyphomycosis caused by *Corynespora cassiicola* in the database PubMed (https://pubmed.ncbi.nlm.nih.gov/) and those retrieved from the literature. The data were summarised according to sex, age, occupation, geographical area, underlying diseases, trauma history, erroneous diagnosis, authors, date, references, duration of diagnosis, disease site, clinical manifestations, laboratory tests, strain identification methods, antifungal treatment, outcomes, and *in vitro* susceptibility results.

3. Results

3.1. Morphological characteristics

Pathology examination of PAS-stained sections revealed the presence of hyphae with septa (red arrow, Figure 2). The biopsy grew mycelial colonies after seven days of culture on PDA, and the growth was much better at 26 °C than at 37 °C. At 26 °C, the

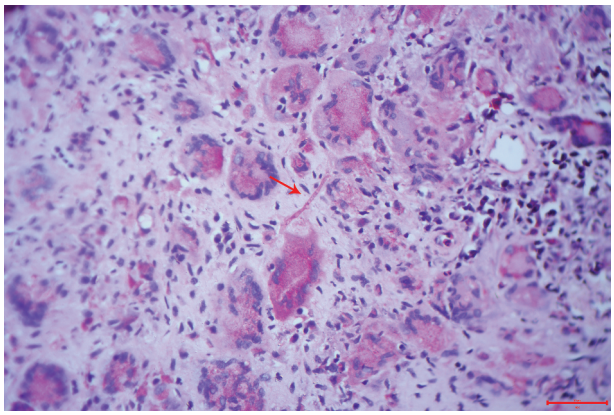

Figure 2. PAS staining revealing hyphae with septa (red arrow, 400× magnification).

mycelium growth rate of *C. cassiicola* was fast, and the average mycelium growth rate was about 5 mm/d, while at 37 °C, the mycelium growth rate was slow, with almost no growth. The colonies have a white, velvety, or somewhat floccose surface (Figure 3a), becoming greyish brown from the central zone, and a dark brown reverse (Figure 3b) at 26 °C and finely villose, greyish at the central zone at 37 °C. The isolate showed optimal growth on SDA in small culture steel rings following seven days of incubation. Microscopically, long, straight, and septate conidiophores and catenate conidia could be found after staining with lactophenol cotton blue (Figure 4a) and 10% KOH (Figure 4b). All the morphological characteristics mentioned above point to the possibility of the pathogen *C. cassiicola*.

3.2. Molecular analysis

The amplified PCR (polymerase chain reaction) products of the ITS, *BT2*, and D regions were sent to a commercial service (Shanghai Majorbio Biopharm Technology Co., Ltd.) for sequencing, and the determined sequences were compared with BLASTn to confirm the identification. The isolate was finally confirmed as *C. cassiicola* with a maximum identity of 99% by the ITS regions (accession No. ON713481), 99% by the *BT2*

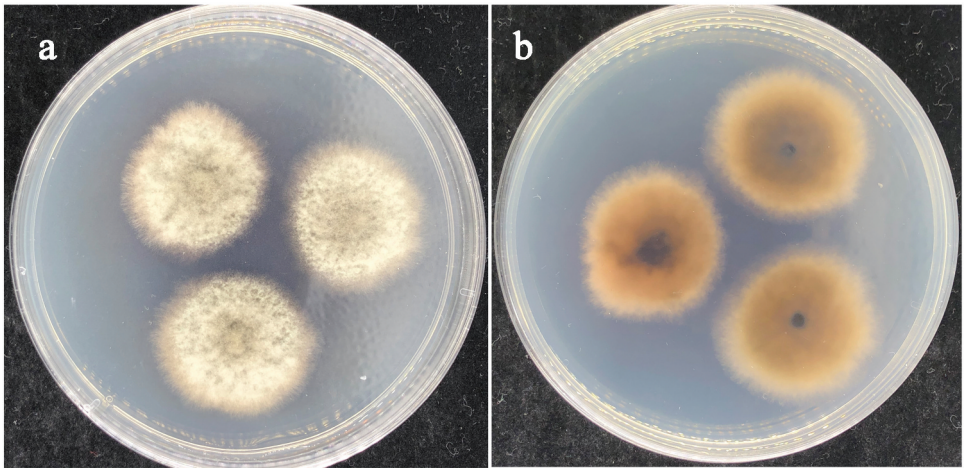

Figure 3. Colonies of isolates grown on PDA were cultured at 26 °C for seven days. (a) Obverse. (b) Reverse.

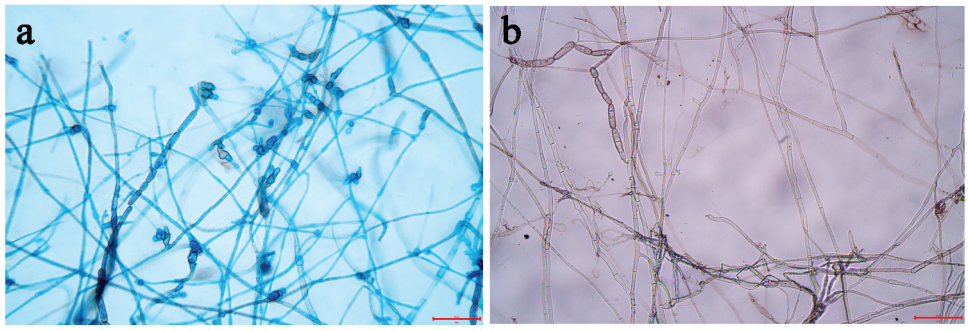

Figure 4. Long, straight and septate conidiophores and catenate conidia. (a) lactophenol cotton blue stain, 400× magnification. (b) 10% KOH stain, 400× magnification.

regions (accession No. ON713483), and 99% by the D regions (accession No. ON713482). Sequences of *C. cassiicola* G16 KP734242, *C. ligustri* MH782602, *C. cassiicola* MH605273, *C. cambrensis* MF428394, *C. submersa* NR 170017, *C. thailandica* MK047455, *C. smithii* MN121566, *C. citricola* MN840557, *C. encephalarti* MK876383, *C. torulosa* KF777154, *C. pseudocassiicola* MH327794, *C. lignicola* NR 170018, *C. olivacea* JQ044429, and *C. proliferata* FJ852596 were used to construct a phylogenetic tree (Figure 5). The result shows that the isolate (red arrow, Contig 1) falls into the cluster of *C. cassiicola* species within the same clade as *C. ligustri* and *C. cambrensis* and confirms that the isolate belongs to the species *C. cassiicola*.

3.3. In vitro *antifungal susceptibility test*

The *in vitro* susceptibility of the isolate to nine common antifungal drugs was tested, and MIC (minimum inhibitory concentration) was defined as the lowest concentration at which growth was 100% inhibited in AmB and 50% inhibited in the other eight drugs. The MIC results were as follows: fluconazole 0.5 μg/mL, itraconazole ≤0.016 μg/mL, voriconazole 0.008 μg/mL, posaconazole ≤0.008 μg/mL, caspofungin 0.25 μg/mL, micafungin 0.016 μg/mL, anidulafungin ≤0.016 μg/mL, amphotericin B 1 μg/mL, and 5-fluorocytosine 2 μg/mL.

3.4. *Literature review results*

A total of eight patients were obtained who were diagnosed with phaeohyphomycosis caused by *C. cassiicola* and reported in nine articles (Mahgoub 1969; Huang et al. 2010; Lv et al. 2011; Yamada et al. 2013; Yan et al. 2016; Arango-Franco et al. 2018; Wang et al. 2018, 2019; Xie et al. 2018) in the PubMed database from its inception until September 2022, and our patient is the ninth case in the world. The epidemiological characteristics of the nine patients are summarised in Table 1, the clinical data in Table 2, the treatment and outcomes in Table 3, and the *in vitro* susceptibility results in Table 4.

4. Discussions

Corynespora cassiicola belongs to Deuteromycota, Hyphomycetes, Hyphomycetales, Dematiaceae, and *Corynespora*, which is the earliest one found among 169 species of *Corynespora* and the one with the widest range of hosts. This organism can infect plant leaves, stems and roots, nematode cysts, and human skin (Dixon et al. 2009). It was first isolated from rubber trees in Sierra Leone in 1936 and was first reported to cause human infection in Sudan in 1969 (Mahgoub 1969). To the best of our knowledge, there have been only eight patients previously reported to date (Mahgoub 1969; Huang et al. 2010; Lv et al. 2011; Yamada et al. 2013; Yan et al. 2016; Arango-Franco et al. 2018; Wang et al. 2018, 2019; Xie et al. 2018), and our patient is the ninth case infected by *C. cassiicola* and the third case of invasive facial infection. In this study, we sought to retrospectively analyse nine patients with phaeohyphomycosis caused by *C.*

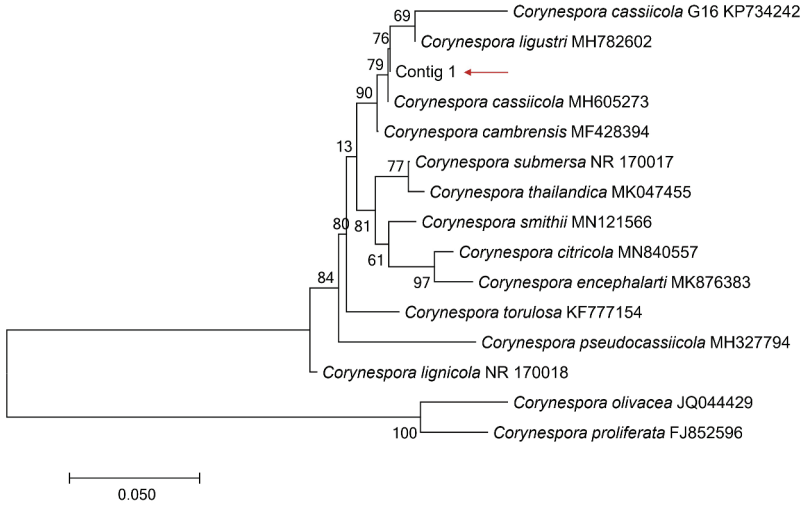

Figure 5. Phylogenetic tree was generated using sequences of 14 related reference fungi.

Table 1. Epidemiological characteristics of patients with phaeohyphomycosis caused by *Corynespora cassiicola*.

Case	Sex	Age (years)	Occupation	Geographical area	Underlying diseases	Trauma history	Erroneous diagnosis	Date	References
P1	M	ND	Farmer	Sudan	ND	ND	ND	1969	Mahgoub (1969)
P2	F	69	Farmer (cultivated papaya, banana and betel nut)	Taiwan China	Diabetes mellitus; iatrogenic cushing syndrome	Did not notice	Cellulitis	2010	Huang et al. (2010)
P3	M	57	Farmer (cultivated eggplants, cucumbers and tomatoes)	Mainland China	Healthy	Excessive scratching	Dermatitis; vasculitis	2011	Lv et al. (2011)
P4	M	76	Farmer (growed a variety of plants)	Japan	Not a contact lens wearer	NO	Herpetic keratitis	2013	Yamada et al. (2013)
P5	F	37	Freelance worker	Mainland China	*CARD9* mutation: p. L64fsX59 and p. D274fsX60	Denied	Sarcoidosis; drug eruption	2016	Yan et al. (2016); Arango-Franco et al. (2018)
P6	M	76	Denied histories of plant cultivation	Mainland China	COPD; hypertension; acute heart failure	Denied	Not taken seriously	2018	Wang et al. (2018)
P7	F	8	Born in a rural area	Colombia	Biallelic mutations in *CARD9*	ND	Fungal vs parasitic infection	2018	Xie et al. (2018)
P8	M	84	Retired (living in an agriculture county)	Taiwan China	COPD (inhaled corticosteroids)	NO	Cellulitis	2019	Wang et al. (2019)
P9	M	17	Student (living in an agriculture county)	Mainland China	Healthy	Recurrent epistaxis	Not taken seriously	2020	This study

P, patient; M, male; F, female; ND, no data; COPD, chronic obstructive pulmonary disease.

Table 2. Clinical data of patients with phaeohyphomycosis caused by *Corynespora cassiicola*.

Case	Duration of diagnosis	Disease site	Clinical manifestations	Laboratory tests
P1	6 months	Foot	Swollen	ND
P2	1 month	Bilateral forearms; dorsal aspect of both hands	Erythematous change; purulent discharge; painful sensation	Leukocyte count 10,100/μL
P3	2 months	Both legs	Indurated plaques, nodules, erosions and ulcers	Leukocyte count 7,800/μL (55.1% granulocytes); ESR 4 mm/h
P4	ND	Cornea of right eye	A foreign-body sensation in the right eye	Slit-lamp examination showed a corneal epithelial defect and an oval zone of infiltration in the lower half of the right corneal stroma with an irregular border
P5	2 years	Face	Swollen and painful plaques	Leukocyte count 3,990/μL (58.8% granulocytes); ESR 49 mm/h; CRP 49.6 mg/L; CD4+/CD8+ T-cell count 13.8%; G test 5,000 pg/mL
P6	2 months	Right leg	Ulcers scattered as multifocal lesions with purulent discharge	Leukocyte count 18,000/μL (92% granulocytes); CRP 15 mg/L; PCT 0.74 ng/mL
P7	4 years	Left side of face	A large, indurated, foul-smelling, and verrucous ulcerated lesion with extensive necrosis and crusting	Serum GM test positive; normal to mild leukocyte count; normal absolute counts of lymphocytes and granulocytes
P8	4 months	Right dorsal hand	A painful ulcer with discharge	Leukocyte count 7,000/μL (80.1% granulocytes); CRP 18.7 mg/L
P9	2 months	Nasal cavity	Nasal dorsum bulge with peculiar smell	Leukocyte count 6,800/μL (59.7% granulocytes, 0.7% eosinophils); ESR 4 mm/h; CRP 13.49 mg/L; PCT 0.024 ng/mL; CD4+/CD8+ T-cell count 1.08; G test 242.9 pg/mL; Ig E 364 IU/mL

P, patient; ND, no data; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; G, 1,3-β-D-glucan; PCT, procalcitonin; GM, galactomannan; Ig, immunoglobulin.

cassiicola in humans, with a focus on the epidemiological characteristics, clinical data, treatment and outcomes, and *in vitro* antifungal susceptibility results.

Given that *C. cassiicola* is a phytopathogen, most patients were found to be either farmers or lived in agricultural areas. This also explains why physical contact facilitated by a breach in the host's physical barrier may pave the way for the initiation of infection. Our literature review found that infections all occurred in exposed parts of the body in nine patients, including 44.4% (4/9) on the face, 33.3% (3/9) on the lower limbs, and 22.2% (2/9) on the upper limbs. In line with our theory, our patient had recurrent epistaxis, and he may have been colonised via inhalation of fungal spores. Of note, traumas were not described in published cases, which may indicate that either such incidences have been overlooked or that patients may have not noticed them. Moreover, all patients were from Asian, African, and Latin American countries, of whom 66.7% (6/9) were from Taiwan China and mainland China. The reason accounting for this phenomenon is related to the fact that *C.*

Table 3. Treatment and outcomes of patients with phaeohyphomycosis caused by *Corynespora cassiicola*.

Case	Strain identification methods	Antifungal treatment	Outcomes
P1	Isolated in culture	ND	ND
P2	Sequence analysis of ITS regions	Oral IZ 200 mg twice/d → A total AmB dose of 1,000 mg	Recovered completely
P3	Sequence analysis of ITS regions	Oral TBF 250 mg/d plus topical wet dressing of 5% povidone iodine for 1 month	All resolved
P4	Sequence analysis of ITS regions	Topical 1% VOR and 0.1% MF/h plus 5% pimaricin ointment 5 times/d for 4 months, 400 mg/d IV VOR for 1 month plus 400 mg/d oral VOR for 1 month	No reactivation of the *Corynespora* infection observed for 6 months
P5	Sequence analysis of ITS regions	IV AmB and oral IZ 400 mg/d plus TBF 500 mg/d	Resistant to treatment
P6	Gene analysis of the isolate	IV VOR for 3 days → Oral VOR for 2 months plus lesions washed with potassium permanganate (1:5,000) once/d	All lesions healed completely
P7	Sequence analysis of ITS and D regions	IV AmB for 1 month → Oral VOR 10 mg/(kg·d) for 10 weeks → IV AmB 1 mg/(kg·d) for 1 month → IV VOR 6 mg/(kg·d) plus CAS 50 mg/(m^2·d) for 2 weeks → Oral VOR for 12 weeks → IV LAmB 5 mg/(kg·d) plus liquid oral PZ 20 mg/(kg·d) for 1 month → Oral PZ plus TBF 125 mg/d → IV LAmB 5 mg/(kg·d) plus PZ 20 mg/(kg·d) for 10 days → Oral PZ plus TBF → IV LAmB	Continued to deteriorate
P8	Sequence analysis of ITS regions	Oral IZ 100 mg once/d for 16 days → Oral VOR 50 mg once/d for 2 weeks→ Oral TBF for 12 weeks	The ulcerative lesion completely resolved
P9	Sequence analysis of ITS, *BT2* and D regions	Oral IZ 200 mg twice/d for 2 weeks → IV VOR 200 mg twice/d for 3 days → IV LAmB 50 mg gradually escalated to 100 mg once/d plus nasal cavity washed with LAmB 20 mg mixed with 5% glucose solution 50 mL for 1 month → Oral VOR 200 mg twice/d	No reactivation found and still under follow-up observation

P, patient; ITS, internal transcribed spacer; β-tubulin, *BT2*; ND, no data; IZ, itraconazole; AmB, amphotericin B; TBF, terbinafine; VOR, voriconazole; MF, micafungin; IV, intravenous; CAS, caspofungin; LAmB, amphotericin B liposomal; PZ, posaconazole.

Table 4. *In vitro* susceptibility results of patients with phaeohyphomycosis caused by *Corynespora cassiicola* (μg/mL).

Case	KZ	MZ	EZ	BZ	FZ	IZ	VOR	PZ	TBF	BTF	CAS	MF	AND	AmB	NT	5-FC
P1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P2	ND	ND	ND	ND	ND	1	ND	ND	ND	ND	ND	ND	ND	0.5	ND	ND
P3	1	ND	2	16	8	8	ND	ND	0.125	0.25	ND	ND	ND	ND	1	ND
P4	ND	0.5	ND	ND	16	0.12	≤0.015	ND	ND	ND	ND	≤0.03	ND	0.06	ND	32
P5	ND	ND	ND	ND	ND	4	0.25	ND	0.125	ND	16	ND	ND	2	ND	ND
P6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P7	ND	ND	ND	ND	ND	ND	0.75	1	ND	ND	ND	ND	ND	0.125	ND	ND
P8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P9	ND	ND	ND	ND	0.5	≤0.016	0.008	≤0.008	ND	ND	0.25	0.016	≤0.016	1	ND	2

P, patient; ND, no data; KZ, ketoconazole; MZ, miconazole; EZ, econazole; BZ, bifonazole; FZ, fluconazole; IZ, itraconazole; VOR, voriconazole; PZ, posaconazole; TBF, terbinafine; BTF, butenafine; CAS, caspofungin; MF, micafungin; AND, anidulafungin; AmB, amphotericin B; NT, nystatin; 5-FC, 5-fluorocytosine.

cassiicola is mainly distributed in tropical and subtropical climate zones (Qi et al. 2009), and the above-mentioned geographical areas have a large population engaged in agricultural production. Additionally, patients were mostly male (male-to-female ratio, 2:1), which might be due to the division of labour in agricultural production activities.

Phaeohyphomycosis usually occurs in patients with in-born or acquired immunosuppression. Among the nine patients in this study, three experienced different underlying diseases or a history of using immunosuppressive drugs, and two patients were confirmed to have mutations in *CARD9* (note that three of the patients in the current pooled analysis did not have underlying conditions). *CARD9* is a bridge connecting innate and adaptive immunity, which can induce the body to produce proinflammatory cytokines to defend against the invasion of microorganisms, such as fungi (Drewniak et al. 2013; Corvilain et al. 2018; Zhong et al. 2018). *CARD9* deficiency predisposes healthy individuals to opportunistic fungal infections, and it was first linked to chronic mucocutaneous candidiasis in 2009 by Glocker et al. (2009), with subsequent reports mainly associated with *Candida* species and dermatophyte infections (Lanternier et al. 2013). Yan et al. (2016) linked *CARD9* deficiency with susceptibility to opportunistic *C. cassiicola* infection. Notably, Arango-Franco et al. (2018) found that many patients with *CARD9* deficiency showed mild or marked elevation of serum eosinophil count, and at the same time, Quan et al. (2019) pointed out that elevated serum IgE levels were somewhat correlated with *CARD9* deficiency. Hence, when a patient is suspected of *CARD9* deficiency but not eligible for genetic testing, the

change in the above two indicators can first be considered.

The clinical manifestations were mainly non-specific inflammatory reactions, such as redness and hyperaemia, swelling, warmth, and pain, which made clinical diagnosis more difficult. For this reason, the duration from the first symptom/sign to a confirmed diagnosis ranged from one month to four years (median time, three months), and almost all patients had experienced an erroneous diagnosis. The morphological findings reported so far for *C. cassiicola* are perplexing, and accurate and rapid identification can be achieved by sequencing barcoding genes.

Through the *in vitro* susceptibility results summarised in Table 4, we found that *C. cassiicola* was sensitive to most antifungal drugs. The treatment and outcomes are summarised in Table 3. Except for one case (P1) with no data and our patient (P9) still under follow-up observation, five of the other seven patients were successfully cured, and two patients (P5 and P7) showed poor outcomes, manifested as treatment resistance or continued deterioration. Among the five patients who were successfully cured, two (P4 and P6) were treated with voriconazole, two (P3 and P8) with terbinafine, and one (P2) with AmB; at the same time, three (P3, P4, and P6) also received topical antifungal irrigation treatment. Our patient (P9) was treated with intravenous LAmB followed by oral voriconazole combined with topical antifungal irrigation, and the condition was effectively controlled.

It has been recommended that itraconazole or voriconazole could be used for the treatment of phaeohyphomycosis (Arendrup et al. 2014). However, we found that itraconazole might not be an appropriate choice for *C. cassiicola* infection because among the three patients treated with itraconazole (P2, P8, and P9) no obvious improvement in outcome was noted. According to the guidelines (Gilbert 2014), voriconazole is recommended as the first-line treatment for most filamentous fungi. Since voriconazole could be well distributed in the skin and soft tissues, in this study, the treatment of corneal and subcutaneous infections caused by *C. cassiicola* all achieved satisfactory results. Notably, pruritic skin rash and photosensitivity will occur in less than 10% of patients treated with voriconazole (Lat and Thompson 2011). In this study, one patient (P8) developed itchy erythematous papules on the neck and upper extremities after oral voriconazole, which disappeared after discontinuation. Similarly, our patient (P9) developed intolerable visual impairment after intravenous voriconazole, which disappeared after replacement with intravenous LAmB. After reviewing the drug instructions and related literature, much clinical evidence showed that visual impairment was a common adverse reaction of voriconazole, and it was usually reversible. The symptoms could be alleviated or disappeared by adjusting the usage and dosage, such as replacing intravenous administration with oral administration. Based on this, considering that the oral bioavailability of voriconazole could also reach 96%, we chose oral voriconazole for consolidation therapy, and no visual impairment recurred.

Despite attempts at various antifungal regimens, the two patients (P5 and P7) did not achieve satisfactory results. By summarising the commonalities of these two cases, we found that they were both invasive facial infections with *CARD9* deficiency, and *CARD9* deficiency should be the leading cause of host insensitivity to antifungal therapy. Currently, there are few studies on the treatment of *CARD9* deficiency by immune reconstitution, and as reported, exogenous G-CSF (granulocyte colony-stimulating factor) and GM-CSF (granulocyte-macrophage colony-stimulating factor) adjuvant immunotherapy are likely options for future research (Gavino et al. 2014; Celmeli et al. 2016).

In conclusion, we reported one case of phaeohyphomycosis caused by *C. cassiicola* and systematically reviewed eight cases reported in the English literature. When a patient suffers from a persistent infection, the possibility of opportunistic dematiaceous fungal infection should be considered, and the history of physical trauma and plant exposure should be investigated in detail. Microscopic examination in tandem with molecular methods can precisely identify the pathogen at the species level. Voriconazole, terbinafine, and AmB, either alone or in combination, are effective in the treatment of *C. cassiicola* infection. However, when the above antifungal drugs are ineffective, *CARD9* deficiency should be considered.

Disclosure statement

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References

Arango-Franco CA, Moncada-Velez M, Beltran CP, Berrio I, Mogollon C, Restrepo A, Trujillo M, Osorio SD, Castro L, Gomez LV, et al. 2018. Early-onset invasive infection due to *Corynespora cassiicola* associated with compound heterozygous *CARD9* mutations in a Colombian patient. J Clin Immunol. 38(7):794–803. doi:10.1007/s10875-018-0549-0.

Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, European Society of Clinical M, Infectious Diseases Fungal Infection Study G, European Confederation of Medical M. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. Clin Microbiol Infect. 20 (Suppl 3):76–98. doi: 10.1111/1469-0691.12360.

Bongomin F, Gago S, Oladele RO, Denning DW. 2017. Global and multi-national prevalence of fungal diseases-estimate precision. J Fungi (Basel). 3(4):57. doi:10.3390/jof3040057.

Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. 2012. Hidden killers: Human fungal infections. Sci Transl Med. 4(165):165rv113. doi:10.1126/scitranslmed.3004404.

Celmeli F, Oztoprak N, Turkkahraman D, Seyman D, Mutlu E, Frede N, Koksoy S, Grimbacher B. 2016. Successful granulocyte colony-stimulating factor treatment of relapsing *Candida albicans* meningoencephalitis caused by *CARD9* deficiency. Pediatr Infect Dis J. 35(4):428–431. doi:10.1097/INF.0000000000001028.

CLSI. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: Approved standard—second edition. In: CLSI document. Wayne, PA: Clinical and Laboratory Standards Institute; pp. M38–A2.

Corvilain E, Casanova JL, Puel A. 2018. Inherited *CARD9* deficiency: Invasive disease caused by ascomycete fungi in previously healthy children and adults. J Clin Immunol. 38 (6):656–693. doi:10.1007/s10875-018-0539-2.

Dixon LJ, Schlub RL, Pernezny K, Datnoff LE. 2009. Host specialization and phylogenetic diversity of *Corynespora cassiicola*. Phytopathology. 99(9):1015–1027. doi:10.1094/PHYTO-99-9-1015.

Drewniak A, Gazendam RP, Tool AT, van Houdt M, Jansen MH, van Hamme JL, van Leeuwen EM, Roos D, Scalais E, de Beaufort C, et al. 2013. Invasive fungal infection and impaired neutrophil killing in human *CARD9* deficiency. Blood. 121(13):2385–2392. doi:10.1182/blood-2012-08-450551.

Gavino C, Cotter A, Lichtenstein D, Lejtenyi D, Fortin C, Legault C, Alirezaie N, Majewski J, Sheppard DC, Behr MA, et al. 2014. *CARD9* deficiency and spontaneous central nervous system candidiasis: Complete clinical remission with GM-CSF therapy. Clin Infect Dis. 59(1):81–84. doi:10.1093/cid/ciu215.

Ghazanfari M, Arastehfar A, Davoodi L, Yazdani Charati J, Moazeni M, Abastabar M, Haghani I, Mirzakhani R, Mayahi S, Fang W, et al. 2021. Pervasive but neglected: A perspective on COVID-19-associated pulmonary mold infections among mechanically ventilated COVID-19 patients. Front Med. 8:649675. doi: 10.3389/fmed.2021.649675.

Gilbert DN. 2014. Sanford guide to antimicrobial therapy 2016 (spiral edition). The Sanford Guide To Antimicrobial Therapy. UK: Antimicrobial Therapy. p. 1–254.

Glocker EO, Hennigs A, Nabavi M, Schaffer AA, Woellner C, Salzer U, Pfeifer D, Veelken H, Warnatz K, Tahami F, et al. 2009. A homozygous *CARD9* mutation in a family with susceptibility to fungal infections. N Engl J Med. 361 (18):1727–1735. doi:10.1056/NEJMoa0810719.

Hong N, Chen M, Fang W, Al-Hatmi AMS, Boekhout T, Xu J, Zhang L, Liu J, Pan W, Liao W. 2017. Cryptococcosis in HIV-negative patients with renal dialysis: A retrospective analysis of pooled cases. Mycopathologia. 182(9–10):887–896. doi:10.1007/s11046-017-0163-3.

Huang HK, Liu CE, Liou JH, Hsiue HC, Hsiao CH, Hsueh PR. 2010. Subcutaneous infection caused by *Corynespora cassiicola*, a plant pathogen. J Infect. 60(2):188–190. doi:10.1016/j.jinf.2009.11.002.

Isa-Isa R, Garcia C, Isa M, Arenas R. 2012. Subcutaneous phaeohyphomycosis (mycotic cyst). Clin Dermatol. 30(4):425–431. doi:10.1016/j.clindermatol.2011.09.015.

Jackson BR, Chow N, Forsberg K, Litvintseva AP, Lockhart SR, Welsh R, Vallabhaneni S, Chiller T. 2019. On the origins of a species: What might explain the rise of *Candida auris*? J Fungi (Basel). 5(3):58. doi:10.3390/jof5030058.

Lanternier F, Mahdaviani SA, Barbati E, Chaussade H, Koumar Y, Levy R, Denis B, Brunel AS, Martin S, Loop M, et al. 2015. Inherited *CARD9* deficiency in otherwise healthy children and adults with *Candida* species-induced meningoencephalitis, colitis, or both. J Allergy Clin Immunol. 135 (6):1558–1568. doi:10.1016/j.jaci.2014.12.1930.

Lanternier F, Pathan S, Vincent QB, Liu L, Cypowyj S, Prando C, Migaud M, Taibi L, Ammar-Khodja A, Stambouli OB, et al. 2013. Deep dermatophytosis and inherited *CARD9* deficiency. N Engl J Med. 369(18):1704–1714. doi:10.1056/NEJMoa1208487.

Lat A, Thompson GR 3rd. 2011. Update on the optimal use of voriconazole for invasive fungal infections. Infect Drug Resist. 4:43–53. doi:10.2147/IDR.S12714.

Lv GX, Ge YP, Shen YN, Li M, Zhang X, Chen H, Deng S, de Hoog GS, Liu WD, de Hoog GS. 2011. Phaeohyphomycosis caused by a plant pathogen, *Corynespora cassiicola*. Med Mycol. 49 (6):1–5. doi:10.3109/13693786.2011.553635.

Mahgoub E. 1969. *Corynespora cassiicola*, a new agent of maduromycetoma. J Trop Med Hyg. 72(9):218–221.

Qi Y, Xie Y, Zhang X, Pu J, Zhang H, Huang S, Zhang H. 2009. Molecular and pathogenic variation identified among isolates of *Corynespora cassiicola*. Mol Biotechnol. 41 (2):145–151. doi:10.1007/s12033-008-9109-9.

Quan C, Li X, Shi RF, Zhao XQ, Xu H, Wang B, Wang XP, Hu WG, Cao H, Zheng J. 2019. Recurrent fungal infections in a Chinese patient with *CARD9* deficiency and a review of 48 cases. Br J Dermatol. 180(5):1221–1225. doi:10.1111/bjd.17092.

Revankar SG, Sutton DA. 2010. Melanized fungi in human disease. Clin Microbiol Rev. 23(4):884–928. doi:10.1128/CMR.00019-10.

Wang CH, Chen WT, Ting SW, Sun PL. 2019. Subcutaneous fungal infection caused by a non-sporulating strain of *Corynespora cassiicola* successfully treated with terbinafine. Mycopathologia. 184(5):691–697. doi:10.1007/s11046-019-00393-0.

Wang X, Zhang R, Wu W, Song Y, Wan Z, Han W, Li R. 2018. Impaired specific antifungal immunity in *CARD9*-deficient patients with phaeohyphomycosis. J Invest Dermatol. 138 (3):607–617. doi:10.1016/j.jid.2017.10.009.

Xie Z, Wu W, Meng D, Zhang Q, Ma Y, Liu W, Chen J. 2018. A case of phaeohyphomycosis caused by *Corynespora cassiicola* infection. BMC Infect Dis. 18(1):444. doi:10.1186/s12879-018-3342-z.

Yamada H, Takahashi N, Hori N, Asano Y, Mochizuki K, Ohkusu K, Nishimura K. 2013. Rare case of fungal keratitis caused by *Corynespora cassiicola*. J Infect Chemother. 19 (6):1167–1169. doi:10.1007/s10156-013-0579-8.

Yan XX, Yu CP, Fu XA, Bao FF, Du DH, Wang C, Wang N, Wang SF, Shi ZX, Zhou GZ, et al. 2016. *CARD9* mutation linked to *Corynespora cassiicola* infection in a Chinese patient. Br J Dermatol. 174(1):176–179. doi:10.1111/bjd.14082.

Zhong X, Chen B, Yang L, Yang Z. 2018. Molecular and physiological roles of the adaptor protein *CARD9* in immunity. Cell Death Disease. 9(2):52. doi:10.1038/s41419-017-0084-6.