

Isolation of *Exophiala dermatitidis* from endotracheal aspirate of a cancer patient

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Summary

Exophiala (Wangiella) dermatitidis is a melanised (darkly pigmented) yeast-like organism that has been reported from the environment and wild animals. The organism is a frequent coloniser of lungs of patients with cystic fibrosis and causes occasional disseminated phaeohyphomycosis and fungaemia. *Exophiala dermatitidis* is distributed worldwide, but cerebral cases are restricted to East Asia. We report a case of 54-year-old Qatari female patient with a known history of cancer, suffering from pulmonary disorder. Culture of endotracheal aspirate revealed the growth of *E. dermatitidis* concomitant with *Candida krusei*. The final diagnosis of *E. dermatitidis* and attribution to genotype B was achieved by sequencing the rDNA internal transcribed spacer (ITS) region. The present case concerns a pulmonary colonisation by *E. dermatitidis*, similar to that commonly seen in cystic fibrosis patients. For the detection of *E. dermatitidis* in clinical specimens culturing techniques are required. The patient finally expired with persistent cancer and *C. krusei* fungaemia. Review of literature and listing of *E. dermatitidis* cases published after 1992 shows a sharp increase in clinical cases during the 1990s.

Key words: *Exophiala dermatitidis*, colonisation, pulmonary disorder, cancer patient.

Introduction

Exophiala (Wangiella) dermatitidis is a darkly pigmented yeast-like organism belonging to the ascomycete order Chaetothyriales. It is occasionally found in the environment¹⁻⁷ and reported from wild animals,^{3,4} and may cause infections in both immunocompromised and immunocompetent individuals. *Exophiala dermatitidis* is well known to cause local skin⁸ and disseminated phaeohyphomycosis and fungaemia.^{9,10} This species is distributed worldwide, but nevertheless cerebral cases are restricted to East Asia.¹¹ It is regularly reported from sputum of patients with cystic fibrosis as coloniser of the respiratory system,^{9,12-14} or as causal agent for fungal pneumonia¹⁵ and pulmonary phaeohyphomycosis.¹⁶ In the present study, we report a respiratory tract coloni-

sation of *E. dermatitidis* in a cancer patient suffering from *Candida krusei* fungaemia and pulmonary disorder. A full report regarding this case, and review of the recent clinical literature on *E. dermatitidis* is presented.

Patient

Patient was 54-year-old female with diabetes mellitus, hypertension, bronchial asthma, cervical cancer with urinary bladder and colonic metastasis with radiotherapy-induced rectal stricture. She was admitted to Hamad Medical Corporation with the complaint of fever and constipation for 2 days associated with lower abdominal colicky pain and vomiting. She had a history of a left nephrectomy for 15 years and a nephrostomy inserted in the right kidney 6 weeks earlier. Abdominal examination revealed soft lax abdomen with severe tenderness mainly in the lower abdomen with exaggerated bowel sounds. Rectal examination revealed empty rectum with no blood. White blood cell count was 9.9, erythrocyte sedimentation rate = 65 mm h⁻¹, normal haemoglobin and electrolytes. Random blood sugar was

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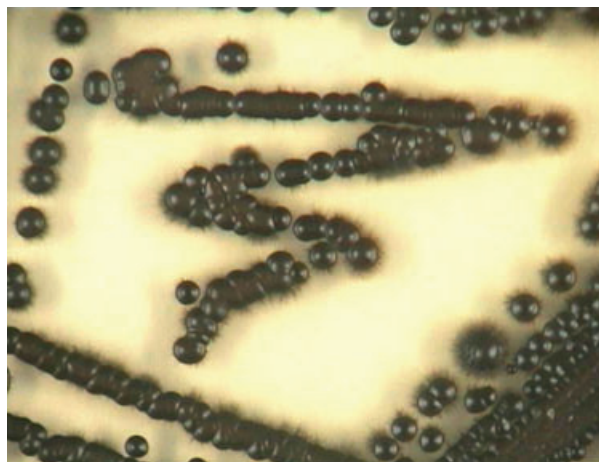
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16.6 mmol with no ketones in the serum. Her chest X-ray revealed minimal bilateral basal infiltrates. Computerized tomography (CT) abdomen revealed no evidence of air fluid level, a small amount of faeces distal to the sigmoid, a small amount of fluid in the para colic gutter more on the left side and solitary right kidney with nephrostomy tube. Ultrasound confirmed the presence of moderate ascites and right pleural effusion. The patient admitted to the intensive care unit as a case of sepsis, she was started empirically on meropenem and ciprofloxacin together with intravenous fluids and inotrops were added later, as the patient became hypotensive. She underwent peritoneal paracentesis that revealed a pussy greenish material, which upon

2 culture yielded *Enterococcus* sp. and *Streptococcus viridans*. Repeated CT scan 5 days after admission revealed free fluid in peritoneum and air under the diaphragm. The patient's level of consciousness deteriorated and she went into deep coma. Intravenous fluconazole was first started at 400 mg, then 200 mg daily with extra dose of 200 mg after each haemodialysis, this regime was continued for 7 days. On day 8 the patient started to develop ventilator-associated pneumonia, on day 12 the patient become stable haemodynamically and inotrops were stopped. However, fungal cultures of endotracheal tube aspirate revealed profuse growth of *E. dermatitidis* with concomitant *C. krusei*. Blood cultures revealed the presence of *C. krusei* repeatedly. It was not possible to further incubate the blood cultures to detect the slow-growing *E. dermatitidis* as the fast-growing *C. krusei* overgrew any organism in the culture within the first 48 h. Two weeks after admission she started to have fever, conventional amphotericin B was started at a dose of 30 mg every other day, 2 days later it was increased to 40 mg every other day, then increased to 50 mg every other day. Seven days after initiation of amphotericin B, intravenous fluconazole 400 mg daily was added (the cumulative amphotericin was 390 mg). The patient deteriorated for the next few days and was put back on inotrops but finally expired with persistent cancer and *C. krusei* fungaemia.

Fungal culture

Endotracheal tube aspirates of the patient was transferred to the Microbiology Laboratory and processed within 2 h of specimen collection. The specimen was inoculated onto two sets of Sabouraud dextrose agar + 40 U ml⁻¹ streptomycin and 20 U ml⁻¹ penicillin (SDA + SP), SDA (no antibiotics), and brain heart infusion + 40 U ml⁻¹ streptomycin and 20 U ml⁻¹ penicillin. Each plate received 0.3 ml of specimen distri-



COLOUR FIG.

Figure 1 Black colonies of *Exophiala dermatitidis* grown for 7 days on Sabouraud dextrose agar medium at 37 °C.

buted into four drops; one set of plates was incubated at room temperature and the other at 37 °C. Colonies of the first organism appeared within 48 h, were soft and creamy whitish. The organism was purified using SDA-SP and identified using a Vitek II instrument (Biomérieux, Marcy 1 Étoile, France) with its corresponding ID card. The result was compatible with an 'excellent' identification for *C. krusei*. The second organism was slow growing appeared upon prolonged incubation up to 10 days. Colonies were soft black grew at room temperature and 37 °C (Fig. 1). The mycological identification of the second type of black yeast-like colonies was performed based on molecular methods at Centraalbureau voor Schimmelcultures (Utrecht, the Netherlands). Molecular identification was carried out by analysing the rDNA internal transcribed spacer (ITS) sequencing performed according to methods outlined by Matos *et al.* [3]. This approach showed unequivocally that our isolate was *E. dermatitidis* genotype B.¹⁷ Antifungal susceptibility using Etest (Solna, Sweden) for the activity of conventional agents against the present *E. dermatitidis* strain, revealed that our strain was sensitive to amphotericin B, itraconazole and ketaconazole with minimal inhibitory concentration (MIC) 0.25, 0.094 and 0.125 µg ml⁻¹, respectively, and resistant to flucytocine and fluconazole with MICs of <32 and 192 µg ml⁻¹ respectively. All investigations were performed under safety conditions (class II) in order to minimise contamination with airborne micro-organisms.

Discussion

Exophiala dermatitidis is a darkly pigmented yeast-like fungus, because of the presence of dihydroxynaphtha-

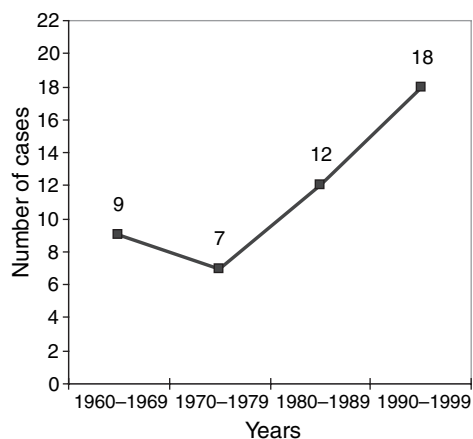


Figure 2 Cases of *Exophiala dermatitidis* reported in the literature for the period of 1960–1999. Data were recorded by carefully scrutinised references for single case reports. Then expanded this initial review by a MEDLINE search using the key word: *Exophiala dermatitidis*.

lene melanin.¹⁸ Production of melanin has been associated with virulence in diverse micro-organisms. Although melanin decreases the host cell stimulation by binding to amphotericin B,¹⁹ *in vitro* experiments failed to detect any differences in MIC between melanised and non-melanised *E. dermatitidis*.²⁰ The natural habitat of *E. dermatitidis* is still insufficiently known. It was recently shown to be abundant in public steam baths³ and water reservoirs.²¹ However, this organism is recognised with increasing frequency as a cause of human disease. A critical review of the literature from the year 1960 regarding the confirmed cases up to 1992 was published by Matsumoto *et al.* [22] and because that review additional cases of various clinical types have been reported from diverse parts of the world. Cases published after 1992 up to 1999 show a sharp increase in clinical cases published during 1990s (Fig. 2).

Invasive disease caused by *E. dermatitidis* is called phaeohyphomycosis and ranges from cutaneous or subcutaneous infection to systemic dissemination of internal organs. Review of the confirmed cases reported after 1992 (Table 1), suggested that the most common manifestations of *E. dermatitidis* are infections of skin, subcutaneous tissue and the central nervous system. Other, less commonly reported sites of infection include eyes, lymph nodes, nails, peritoneum, lungs and internal organs (Table 1).²²

Colonisation by this organism is more frequent in cystic fibrosis patients.^{14,15} However, alterations in the immune status do influence the progress of infectious

Table 1 List of *Exophiala dermatitidis* cases reported after 1992

Case	Manifestation	Predisposing factor	Reference
1	Cervical lymph node, brain	None	41
2	Peritonitis	CAPD	28
3	Septicaemia	ALL	9
4	Brain abscess	None	42
5	Chronic nodule	Rheumatic arthritis	8
6	Catheter-associated fungaemia	HIV	10
7	Corneal ulceration	None	43
8	Melanonychia	None	44
9	Otitis externa	None	45
10	Keratitis	Cornea transplant	23
11	CSF	None	46
12	Peritonitis	CAPD	29
13	Invasive pulmonary	CF	12
14	Lymphadenitis	ALL	25
15	Invasive stomatitis	ML, neutropenia	27
16	Peritonitis	Immunocompromised	47

CAPD, continuous ambulatory peritoneal dialysis; ALL, acute lymphatic leukaemia; CF, cystic fibrosis; ML, myeloid leukaemia; CSF, cerebrospinal fluid.

disease. Predisposing factors for the human infection with *E. dermatitidis* include solid organ transplant,^{23,24} leukaemia,^{9,25,26} cystic fibrosis,^{12,13} neutropenia,²⁶ AIDS,¹⁰ CAPD^{23,29} and chronic diarrhoea.¹⁷

Because of the distinct neurotropism of *E. dermatitidis*, infections because of this fungus can be life-threatening,¹¹ severe cases are observed almost exclusively in immunocompetent patients in eastern Asia, whereas mainly patients with underlying malignancies or with otherwise impaired immunity are affected outside this region.¹¹

Exophiala is a genus of dematiaceous hyphomycetes whose taxonomy and nomenclature undergo constant revision. *Exophiala dermatitidis* differs from other species of the genus by its maximum growth temperature of 42 °C, absence of utilisation of nitrate and nitrite and the apparent absence of annelides, when observed with light microscopy, leading many authors to place it in a separate genus, *Wangiella*.³⁰ The organism grows slowly on primary isolation media, and because of the pleomorphic life cycle,³¹ the morphological distinction in culture between *E. dermatitidis* and other species of *Exophiala* can be difficult.³² As was recently shown, ITS1 sequencing is a highly reliable tool for species identification of strains that are phylogenetically closely related to this black yeast species. Final identification of the causative organism and attribution of our strain to genotype B is achieved by sequencing of the rDNA ITS

region of the fungus.³³ *Exophiala dermatitidis* is frequently clinically resistant to conventional antifungal agents.^{10,34} Because of the rarity of the *Exophiala* disease no large-scale controlled analysis of the antifungal agents has been performed. The MICs of fluconazole and 5-fluorocytosine against several *Exophiala* isolates have been reported to vary greatly, while itraconazole and amphotericin B appear to be effective against *Exophiala* isolates *in vitro* at MICs > 1 µg ml⁻¹.^{32,35–37} *In vitro* studies of Johnson *et al.* [38] revealed the potent and fungicidal activities of amphotericin B, itraconazole and voriconazole. This data supports our results in that amphotericin B and itraconazole are potent *in vitro* inhibitors of *E. dermatitidis*. Catheter-associated fungaemia without deep organ involvement has been treated with itraconazole.³⁹ In another case amphotericin B and 5-fluorocytosine were successfully used for treatment,⁹ whereas other investigators⁴⁰ successfully used fluconazole for the treatment. However, if the infection is systemic or it involves the central nervous system, the addition of amphotericin B is required.

When the infection is initiated by traumatic implantation into the skin, the organism remains localised to the site of inoculation, without dissemination to deeper organs. Alternatively, the organism may be acquired by inhalation of the conidia, exceptionally with haematogenous spread to distinct organs. In order to prevent local recurrence of infection caused by *Exophiala*, treatment should include complete surgical excision of the lesions⁸ that are accessible combined with postsurgical administration of antifungal therapy, such as amphotericin B, ketoconazole and itraconazole, has been advocated^{35–37} especially when invasive or systemic infection is present.

The present study comprises the first reported case of clinical isolation of *E. dermatitidis* in the Middle East, regarding this colonisation. The organism was reported to colonise in the respiratory tract of cystic fibrosis patients.^{14,15} In one case, the organism has been reported to cause invasive pulmonary infection in a patient with cystic fibrosis that was successfully treated with amphotericin B followed by voriconazole.¹² Another well-documented case of pulmonary infection with *E. dermatitidis* was reported in a female patient that manifested infiltrates in the left lower lobe with lingual and apical pleural thickening that was successfully treated with amphotericin B and 5-fluorocytosine.¹⁶ The present case represents a pulmonary colonisation by *E. dermatitidis*, which is similar to what is common in cystic fibrosis patients.^{14,15} Pneumonia or other invasive processes may be at risk of this colonisation. The

source of the organism in the present case cannot be determined, because of the critical and complicated situation of the patient in the present study, only a limited number of tests could be performed. Dissemination was not clear and autopsy was not possible in order to confirm any microbial disease, and therefore no postmortem specimens for culture could be obtained. The primary cause of death was probably persistent cancer and fungaemia caused by *C. krusei* as evident from repeated blood cultures, but it is remarkable that *E. dermatitidis* is found additionally. Culturing techniques for isolation of *E. dermatitidis* should be employed when processing specimens from immunocompromised patients. The prolonged incubation of the cultured specimen is necessary if *E. dermatitidis* are to be recovered.

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