Disseminated Emergomycosis in a Person with HIV Infection, Uganda

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We describe emergomycosis in a patient in Uganda with HIV infection. We tested a formalin-fixed, paraffin-embedded skin biopsy to identify *Emergomyces pasteurianus* or a closely related pathogen by sequencing broad-range fungal PCR amplicons. Results suggest that emergomycosis is more widespread and genetically diverse than previously documented. PCR on tissue blocks may help clarify emergomycosis epidemiology.

Emergomycosis is a fungal infection caused by fungi of the newly described genus *Emergomyces*, of the order Onygenales, which includes obligate fungal pathogens, such as *Histoplasma*, *Blastomyces*, and *Paracoccidioides* (*1*). Emergomycosis manifests after dissemination to the lungs and skin; it is associated with 50% mortality. Most cases of emergomycosis have been reported in persons with HIV from South Africa infected with *E. africanus*, the DNA of which has been amplified from soil there (*2*). Emergomycosis from *E. orientalis* or *E. canadensis* infection has been identified in limited geographic areas.

In contrast, *E. pasteurianus* infections have been widely documented in Asia, Europe, and South Africa (Appendix Table 2) (*2*). *E. pasteurianus* infections were first described in 1998 in a patient in Italy with HIV infection and skin lesions (*3*). The isolate was initially placed in the genus *Emmonsia* because of the similarity of the ribosomal large subunit genes. The new genus *Emergomyces* was suggested by Dukik et al. to distinguish fungi that produce small yeasts in host tissues, comparable to *Histoplasma* instead of the adiaspores found in *Emmonsia* (*4*). We report a case of *E. pasteurianus* infection in a patient in Uganda with HIV infection.

A 38-year-old woman from Rwanda sought treatment in Uganda for a 3-month history of disseminated skin lesions, nodules, papules, and ulcers (Figure). A chest radiograph revealed no signs of disease. The woman was living in southwestern Uganda, working as a trader. She reported no travel except for a short visit to Dubai 5 years earlier. She had also been diagnosed with HIV 5 years earlier. She was treated for HIV with zidovudine, lamivudine, and nevirapine. CD4 lymphocyte count was 140 cells/μL. HIV viral load testing was not performed.

A skin biopsy was taken, but fungal isolation was not performed because laboratorians lacked the necessary equipment. As therapy for emergomycosis, experts suggest amphotericin B, which was not available for the patient, followed by oral triazoles (*2*). The patient was started on fluconazole (400 mg 1×/d) for suspected cutaneous cryptococcosis. Histopathology showed narrow budding yeast cells (2–4 μm) (Figure). Because skin lesions increased during 6 weeks of fluconazole and antiretroviral treatments, treatment was changed to itraconazole (400 mg 2×/d). Lesions decreased markedly within 8 weeks, which we considered the key finding suggesting treatment response. No follow-up data are available beyond this point. As reported by Dukik et al., in vitro resistance testing of *Emergomyces* documents activity of itraconazole, voriconazole, and posaconazole, but not fluconazole (*5*).

In Germany, DNA was extracted from the formalin-fixed, paraffin-embedded (FFPE) skin biopsy as previously described (*6*). Fungal DNA was amplified by 2 broad-range

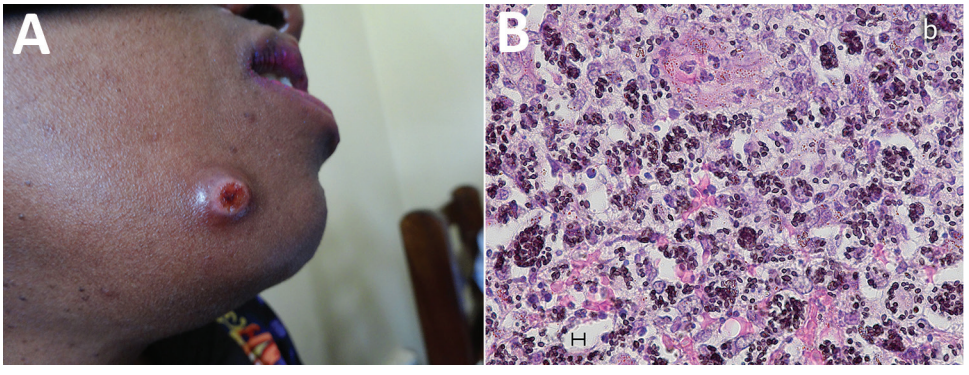

Figure. Imaging from investigation of emergomycosis in a 38-year-old woman from Rwanda with HIV infection living in Uganda. A) Skin lesion on face. B) Histopathology of skin biopsy specimen (Grocott stain) showing multiple budding yeast cells (2–3 μm), mostly in clusters. Scale bar indicates 5 μm.

PCR assays targeting a region of the 28S and the internal transcribed spacer (ITS) 2 regions of the fungal ribosomal RNA genes. Sanger sequencing of the PCR amplicons revealed 365-bp and 273-bp sequences (Appendix, https://wwwnc.cdc.gov/EID/article/25/9/18-1234-App1.pdf). A BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed *Paracoccidioides lutzii* (98.6% pairwise identity) and *E. pasteurianus* (98.9% pairwise identity) to be the closest matches for the 28S and ITS2 amplicons. Because no generally accepted pairwise identity break points for fungal species identification are available and sequence data for the region amplified by the 28S assay are lacking for many fungi, we sequenced the amplicons of the 2 broad-range PCR assays from fungi of the family *Ajellomycetaceae* and of the species *Coccidioides immitis* (Appendix Table 2). Phylogenetic analysis of the concatenated sequences of both broad-range PCR assays suggested that the patient was infected with *E. pasteurianus* or a closely related species (Appendix Figure).

Identification of fungi from pathology blocks may be used to investigate the etiology of mycosis and define endemic regions of fungal pathogens. However, species identification by histopathology is limited and the optimal molecular identification strategy remains to be defined. Amplification of fungal DNA from FFPE tissue is restricted by amplicon length, PCR inhibition, an excess of host DNA, and contaminating fungal DNA (*7*). The broad-range assays we used were introduced to amplify fungal DNA from an excess of host DNA. They have been successfully applied on FFPE tissue before (*6*,*8*).

The ITS2 assay targets a diverse, noncoding region well represented in public databases. However, variable amplicon length (200–300 bp) suggests that detection limits may vary for different fungi and phylogenetic analysis may be impaired. In contrast, the 28S assay amplifies a more conserved coding region (330–350 bp). Whereas identification of a genus may be achieved, species resolution within a genus may not be possible and sequences of this region are underrepresented in public databases (*4*,*6*).

Our results suggest that emergomycosis is more widespread and genetically diverse than previously documented. This case suggests that using broad-range fungal PCR assays with specific PCR assays to target prevalent pathogens may be a successful approach for identifying fungal etiology from pathology blocks and defining the epidemiology of emergomycosis and related infections.

About the Author

Dr. Rooms is a fifth-year resident in dermatology and venereology at the University Hospital of Essen, Germany; she has volunteered as a dermatologist and HIV specialist in Mbarara, Uganda. Her primary interests are tropical dermatological diseases and skin lesions in immunosuppressed patients.

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Appendix

Methods

Handling of the tissue sample

Sections of 5 µm were cut from a pathology block of the skin biopsy. Aliquots of three sections were placed in Eppendorf Biopur tubes (Eppendorf AG, https://corporate.eppendorf.com/en/). The first and last sections were stained by Grocott's methenamine silver stain for microscopy to document the presence of fungal elements in the tissue. This research was approved by the institutional review board at Goethe University in Frankfurt, Germany (375/10).

DNA extraction and PCR assays

DNA extraction and PCR setup were performed in a laminar air flow hood to avoid contamination by ubiquitous fungi. DNA was extracted from an aliquot of 3 cuts (5µm each) after paraffin was removed from the sample by octane. DNA extraction from the FFPE skin biopsy was done using the MasterPure Yeast DNA extraction kit (Biozym, https://www.biozym.com/site/313/biozym.aspx) with some modifications, as previously described (*1*). First, the deparaffinized tissue sections were placed with 550µl of yeast cell lysis buffer into tubes containing silicon carbide sharps (BioSpec Products, https://biospec.com/) and shaken for 60 seconds at 5 m/s using a FastPrep 120 machine (MP Biomedicals, https://www.mpbio.com/) to allow for a mechanical disruption of the tissue and fungal elements in the tissue. Second, the initial incubation step of the extraction kit in lysis buffer was carried out at 90° for 3 hours because previous experiments suggested increased amounts of amplifiable DNA after application of thermal energy (*2*). Next, following the manufacturer's instructions for DNA precipitation, the samples were exposed to 750µl ice cold isopropanol 100% and incubation at –20°C for 60 minutes. DNA was dissolved in 75µl Triton X 0.1% and stored at

4°C until the PCR assay (*1*,*2*). All qPCR assays were performed using an Applied Biosystems (https://www.thermofisher.com/us/en/home/brands/applied-biosystems.html) 7500 real-time instrument as described previously (*1*). All assays were run for 45 cycles. Fungal PCRs were run in duplicates.

An internal amplification control excluded PCR inhibition. Successful DNA extraction was demonstrated by detection of human DNA by qPCR (target 18s rRNA Gene). A specific qPCR assay for *Histoplasma*, targeting a variable region of ribosomal RNA genes conserved in *Histoplasma* (target ITS1 region), failed to amplify DNA from the sample (*3*). Therefore, two broad-range fungal qPCR assays targeting the 28S- (primer 28S10f-28S12r), and ITS2 region (primer 5,8Sf-28S1r) of the ribosomal RNA genes were used. Both assays were designed to amplify a broad range of fungal DNA in human samples (i.e., primer was designed to bind regions conserved among pathogenic fungi but show mismatches with human DNA) (*4*). PCR primers are described in Appendix Table 1. Further information on performing these assays have been reported previously (*1*).

Fungal DNA was amplified by both broad-range PCR assays. Amplicons of the broad-range assays were purified using the ExoSap-IT-kit (USB-products, https://www.thermofisher.com/us/en/home/life-science/pcr/united-states-biochemical-usb.html) and sequenced bidirectionally. Mastermix negative controls and extraction negative controls, performed as described previously, ruled out fungal DNA contamination (*1*).

Phylogenetic Analysis

Fungal DNA of fungi related to *Emergomyces* was extracted as reported for the tissue samples after cultivation on potato dextrose agar (Appendix Table 2). Fungal DNA was amplified using both broad-range PCR assays as reported for the tissue sample and sequenced accordingly. The phylogenetic tree was inferred from the combined 28S and ITS-2 sequences amplified from the tissue block and reference strains using the maximum likelihood method based on the Tamura 3-parameter model (Appendix Figure 1) (*5*). The model was determined based on the model with the lowest Bayesian information scores as determined using the finding model program in MEGA7 (*6*). Bootstrap was run with 1000 resampling.

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Appendix Table 1. Primer used to amplify fungal DNA from FFPE tissue

qPCR	Primer name	Sequence (5′-3′)	Ref
Histoplasma ITS1	Hc-ITS 167f	AACGATTGGCGTCTGAGCAT	3
	Hc-ITS 229r	GAGATCCGTTGTTGAAAGTTTTGA	
Broad-range ITS2	5.8Sf	GTGAATCATCGARTCTTTGAAC	4
	28S1r	TATGCTTAAGTTCAGCGGGTA	
Broad-range 28S	28S10f	GACATGGGTTAGTCGATCCTA	4
	28S12r	CCTTATCTACATTRTTCTATCAAC	

Appendix Table 2. Generated 28S and ITS2 sequences of fungi

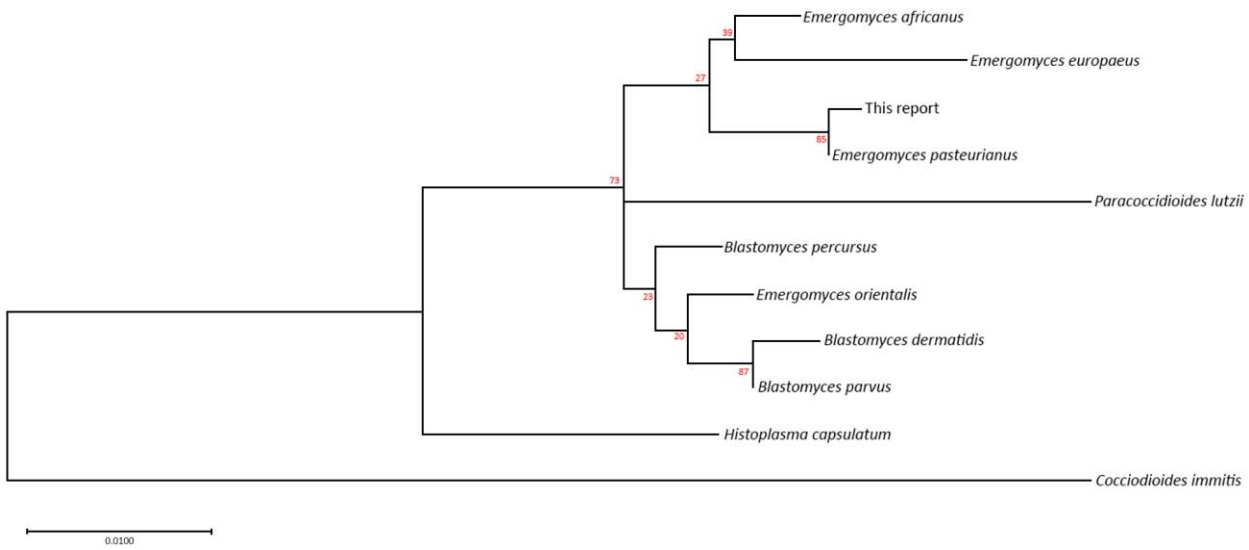
Fungus	Identifier	28 S	ITS2
Histoplasma capsulatum	RKI 12–0644	MH682062	MH682095
Blastomyces percursus	CBS 17–139878	MH682055	MH682088
Blastomyces dermatitidis	ATCC-18188	MH682053	MH682086
Paracoccidioides lutzii	ATCC-MYA-826	MH682061	MH682094
Blastomyces parvus	CBS 139881	MH682054	MH682087
Emergomyces africanus	CBS 136260	MH682056	MH682089
Emergomyces pasteurianus	CBS 101426	MH682058	MH682091
Emergomyces orientalis	CBS 124587	MH682057	MH682090
Emergomyces europaeus	CBS 102456* (GHP 1427, UAMH 10427)	MH682059	MH682092
Coccidioides immitis	RKI 06–0091	MH682060	MH682093
This report	RKI 17–0923	MH686148	MH686147

*obtained from G. Haase (14)

Appendix Table 3. Case reports of emergomycosis caused by *Emergomyces pasteurianus*

Author	Country, Year	Preexisting conditions	Clinical presentation	Accession no. (ITS)
Gori (*7*)	Italy, 1998	AIDS	Disseminated (s)	HF563671
Pelegrin (*8*)	Spain, 2014	AIDS, liver transplantation	Disseminated (s,p)	Not reported
Lavergne (*9*)	France or Georgia, 2017	AIDS	Pulmonary	Not reported
Malik (*10*)	India, 2016	AIDS	Disseminated (s, p)	KR150770
Feng (*11*)	China, 2015	Renal transplantation	Disseminated (s, p)	KT155632 KP730695 KP730694
Tang (*12*)	China, 2015	Glucocorticoid therapy	Disseminated (s)	KP260922
Dukik (*13*)	South Africa, 2017	AIDS	Disseminated (s)	KY195962
This report	Uganda or Rwanda, 2018	AIDS	Disseminated (s)	MH686147

s: skin, p: pulmonary, ITS: internal transcribed spacer



Appendix Figure. Phylogenetic tree of the combined ITS and 28S sequences amplified from the tissue block containing the skin biopsy and sequences from reference strains. A maximum likelihood method using the Tamura 3-parameter substitution model was used based on the lowest Bayesian information scores as determined using the finding model program in MEGA7. Bootstrap was run with 1000 resampling. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 551 positions in the final dataset.