

Black grain eumycetoma due to *Diaporthe ueckerae*. Taxonomical update of previous agents of infections due to *Diaporthe* spp.

Matías S. Cabeza^{a,b}, Abel Gómez^c, Natalia Sasoni^a, Soledad Gamarra^a, Guillermo Garcia-Effron^{a,b,*}

^a Laboratorio de Micología y Diagnóstico Molecular, Cátedra de Parasitología y Micología, Facultad de Bioquímica, Universidad Nacional del Litoral, Ciudad Universitaria, Santa Fe city (Santa Fe), CP 3000, Argentina

^b Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Santa Fe city (Santa Fe), CP 3000, Argentina

^c Clínica de Nefrología Urología y Enfermedades Cardiovasculares, Santa Fe city (Santa Fe), CP 3000, Argentina

ARTICLE INFO

Keywords:

Mycetoma
Eumycetoma
Diaporthe
Black-grain

ABSTRACT

A black-grain eumycetoma due to *Diaporthe ueckerae* in a kidney transplant recipient is presented. The isolate was identified by using the newly available NCBI's curated database (rRNA_typetrains/ITS_RefSeq_Fungi) and the NCBI's GenBank + EMBL + DDBI + PDB + RefSeq database. The isolate's antifungal susceptibility was evaluated. The studied isolate showed low MIC values to the eight tested antifungals. Using this updated database, the identities of previous agents of *Diaporthe* spp. infections were revised.

1. Introduction

Mycetoma is a chronic invalidating infection endemic to tropical regions of the world named “the mycetoma belt”. The World Health Organization recognized these infections as a neglected tropical disease. Mycetoma is named actinomycetoma or eumycetoma when its etiological agent is a bacterium or a fungus, respectively. These diseases usually begin as a result of an accidental puncture or injury with a rural tool or a vegetable debris. Therefore, rural workers with little or no working-protection elements are the most likely to suffer it. Most patients suffering mycetoma are otherwise immunocompetent with no underlying condition [1,2].

Clinical presentations take months to develop and the symptoms includes the so-called mycetoma clinical triad: localized swelling, underlying sinus tracts, and production of grains or compact granules. These grains morphological characteristics are helpful to establish a rapid and provisional diagnosis of the etiological agent. Eumycetoma grains are mostly black or white in color while actinomycetoma grains show a wider palette of colors (ranging from yellowish-white to browns through reds and pinks) [1,2].

The presented case shows some of the described characteristics as: (i) the disease started after an injury with a rural tool (ii) the patient lives in a city located in the rural area of Argentina in the southern limit of the

“mycetoma belt”. This region shares its climate and vegetation (xerophilous) with other endemic areas of mycetoma and (iii) he showed the mycetoma clinical triad of symptoms (with black grains) [1,2]. However, he is not immunocompetent because he is under treatment with immunosuppressants due to his kidney transplant.

2. Case

We are presenting a mycetoma case in a 48-years old male kidney transplant recipient (2012). He was receiving immunosuppressive treatment from the moment of the transplant that includes thymoglobulin, tacrolimus, mycophenolate mofetil and prednisone. He lives in San Cristóbal city (Santa Fe, Argentina) that is located in the rural area of the Chaco plains (latitude and longitude: –30.33333 S and –61.33333 W, respectively). This region is in the southern limit of the so-called “mycetoma belt” and shares its climate and vegetation (xerophilous) with other endemic areas of mycetoma [3,4]. In March 2018, the patient accidentally hurt his foot with a garden tool. This wound evolved into a pseudotumoral lesion that was resected in November 2018 (Fig. 1). In November 2019, the lesion showed soft tissue swelling and the presence of black macroscopic grains and draining sinuses. A second resection was done and black grains were obtained (Fig. 1). At the microscope, the grains were formed by dematiaceous hyphae.

* Corresponding author. Laboratorio de Micología y Diagnóstico Molecular (CONICET), Cátedra de Parasitología y Micología, Facultad de Bioquímica y Ciencias Biológicas (Universidad Nacional del Litoral), Ciudad Universitaria UNL, Santa Fe city (Santa Fe), CP 3000, Argentina.

E-mail addresses: ggarcia@unl.edu.ar, guillermo_garciaeffron@yahoo.com.ar (G. Garcia-Effron).

<https://doi.org/10.1016/j.mmcr.2022.11.001>

Received 2 October 2022; Received in revised form 23 November 2022; Accepted 26 November 2022

Available online 1 December 2022

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Grayish filamentous colonies with dark brown reverse were obtained after 10 days of incubation on Sabouraud Agar at 28 °C. Colonies were formed by sterile dematiaceous septated hyphae (Fig. 1).

Antifungal susceptibility testing was performed by adapting the CLSI M38 3rd ed. Document [5] to the way this fungi growth (hyphae was used as inoculum and the results were read at 96 h). The obtained MIC values were: amphotericin-B 0.5 µg/ml, anidulafungin 0.5 µg/ml, caspofungin 0.5 µg/ml, fluconazole 4 µg/ml, isavuconazole 0.06 µg/ml, itraconazole 0.5 µg/ml, posaconazole <0.015 µg/ml and voriconazole <0.015 µg/ml.

The isolate was identified as *Diaporthe ueckerae* by sequencing of its ITS regions and *TEF1* gene (GeneBank accession number: OL981188, OL999090, respectively). The identity percentages (99.82% and 100%, respectively) were obtained by blasting the sequences against the NCBI's rRNA_typetrains/ITS_RefSeq_Fungi database and against the NCBI's GenBank + EMBL + DDBJ + PDB + RefSeq sequences databases (both updated December 20th 2021), respectively [6]. It has to be noticed that our ITS sequence showed also high percentage of identity with *D. miriciae* and *D. rosae* (98.64% and 98.56%, respectively).

While these studies were conducted, the patient received oral itraconazole (200 mg/day) for two months. This treatment was then switched to fluconazole (200 mg/day) by a general practitioner in his city of origin (without consulting the treating infectious diseases specialist). In November 2021, he returned to the infectious diseases service due to the bad evolution of the lesion and itraconazole therapy was reinstated (200 mg/day for 1 year) (Fig. 1). As of September 2022, the patient shows no signs of infection and the lesion has improved markedly. The treatment will be completed at the end of November 2022.

3. Discussion

Eumycetoma etiological agents belong to different classes (mainly *Sordariomycetes* and *Pleosporalomycetes*) [7]. *Diaporthe* species (Class *Sordariomycetes*) have wide ranges of vegetable hosts and are extensively distributed. These fungal species are phytopathogens, endophytes or saprophytes and rarely as human pathogens. *Diaporthe*'s taxonomy is complex and changing. Many species were originally classified on the basis of its association with their hosts and not on molecular criteria [8]. Human infections have been scantily reported and most of their causative agents were originally identified as *Diaporthe phaseolorum* [9]. Most of these cases were described in renal and heart transplant recipient and in diabetic patients. All but one of these infections involve soft tissues (one described as eumycetoma) (Table 1). In these reported cases, authors classified their strains by blasting their ITS sequences against the non-curated NCBI's database (GenBank + EMBL + DDBJ + PDB + RefSeq database). To confirm these identifications, we took those ITS sequences and retested them but using the newly NCBI's curated database (rRNA_typetrains/ITS_RefSeq_Fungi). We found that all the *D. phaseolorum* isolates were mistakenly identified and they should be reassigned to other highly related species of *Diaporthe* spp. (*D. miriciae*, *D. ueckerae* or *D. rosae*). Thus, all the published *D. phaseolorum* human infection cases (until 2020) were in fact caused by *D. rosae*, *D. miriciae* or *D. ueckerae* (Table 1) [8,10–14]. Remarkably, when our ITS sequence was blasted against the non-curated database it showed the highest identity with *D. phaseolorum* (99.48% identity).

D. ueckerae is one of the etiological agents of a soybean illness named stem canker. It is responsible for more than 15% of the infections of this crop in Uruguay [9]. Interestingly, most of the *Diaporthe* spp. human infections were reported in three of the biggest worldwide producers of soybean (Brazil, India and now in Argentina). It is difficult to infer a

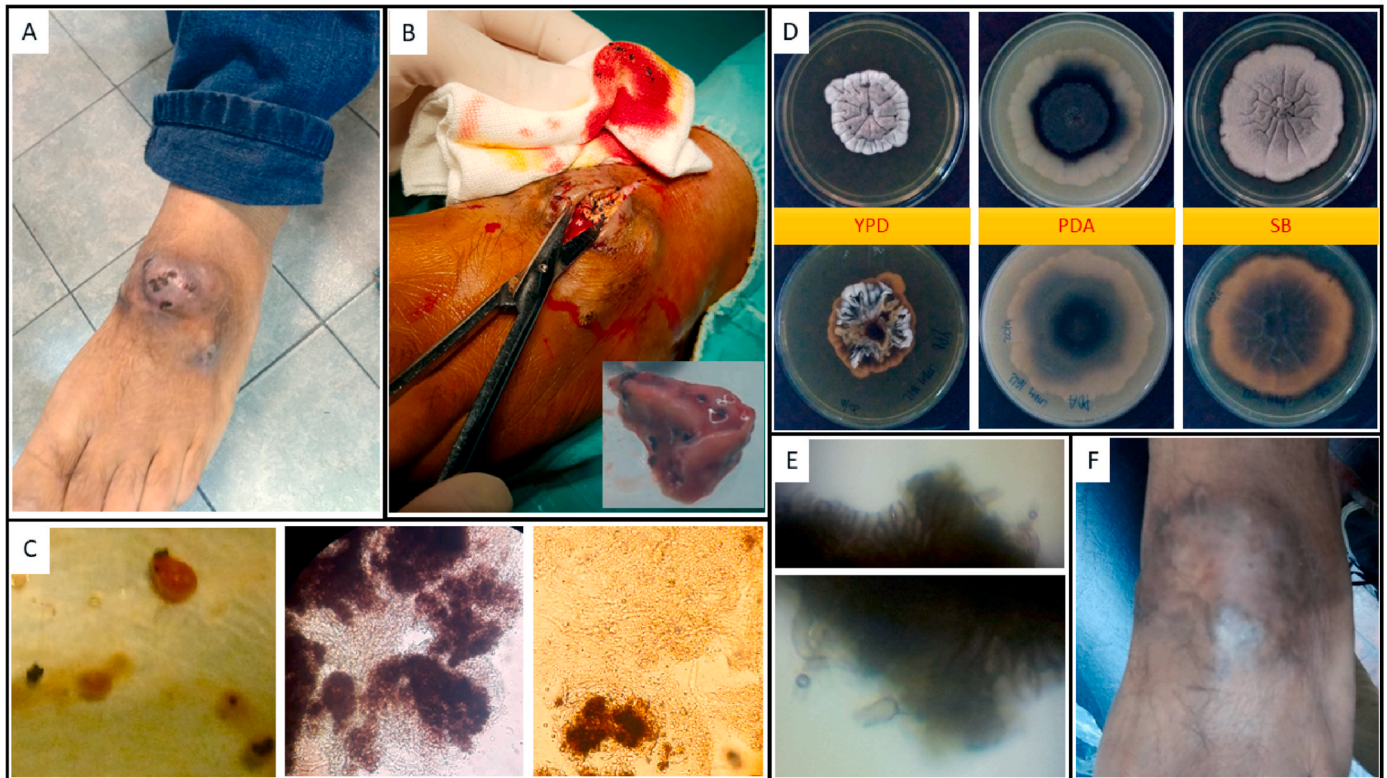


Fig. 1. (A) Pseudotumoral lesion with soft tissue swelling and the presence of black macroscopic grains and draining sinuses. (B) Resection operation (black grains in biopsy). (C) Isolated black grains and dematiaceous hyphae observed at the microscope (100X and 400X). (D) Grayish filamentous colonies with dark brown reverse obtained after 10 days of incubation on Yeast Peptone Dextrose Agar (YPD), Potato Dextrose Agar (PD) and Sabouraud Agar (SB) at 28 °C. (E) Sterile dematiaceous septate hyphae obtained from colonies. (F) Evolution of the lesion (November 2021), before itraconazole treatment was reinstated. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Published cases of human infection caused by *Diaporthe uekeriae* related species.

Reported taxonomical Identification (ITS – original Gene bank accession number)	Taxonomical identification using curated ITS sequence database (accession number/Percent identity) ^a	Age/sex	Underlying disease/immune-suppression	Activity/plant linked with the infection	Clinical presentation	Site	Treatment/evolution ^b	Country/year (ref.)
<i>Diaporthe phaseolorum</i> (<i>Phomopsis phaseoli</i>) (JF896458.1)	<i>D. uekeriae</i> (NR_147543.1/99.10%) <i>D. rosae</i> (NR_172401.1/99.09%)	60/ male	HTLV-1 positive serology	Farmer/ Amaranthus spinosus	Mycetoma	Left forefoot	ITC/clinical remission	French Guiana, Cayenne/2011 [10]
<i>Diaporthe phaseolorum</i> (HF586483)	<i>D. miriciae</i> (NR_147535.1/100%) <i>D. uekeriae</i> (NR_147543.1/99.82%)	43/ male	Renal transplant recipient	Farmer	Soft tissue infection	Right arm and leg	Surgery + ITC (200 mg/day-5 month)/cure.	Brazil/2013 [14]
<i>Diaporthe phaseolorum</i> (KX498068.1)	<i>D. uekeriae</i> (NR_147543.1/99.27%) <i>D. miriciae</i> (NR_147535.1/99.11%)	46/ male	Heart transplant recipient	Not described	Soft tissue infection	Pretibial area of left leg	Surgery + VRC switched to ITC for 7 month/cure	New Zealand/2019 [8]
<i>Diaporthe phaseolorum</i> (MK064209)	<i>D. rosae</i> (NR_172401.1/100%) <i>D. miriciae</i> (NR_147535.1/98.99%)	47/ male	Renal transplant recipient	Clerk	Soft tissue infection	Right leg	Surgery + ITC (400 mg/day 2 month)/clinical remission	India/2019 [11]
<i>Diaporthe phaseolorum</i> (MN788661)	<i>D. rosae</i> (NR_172401.1/99.82%) <i>D. miriciae</i> (NR_147535.1/98.91%) <i>D. uekeriae</i> (NR_147543.1/98.91%)	66/ female	None known.	Hairdresser – living in rural area.	Soft tissue infection	dorsum of the right hand	Surgery + FLC (200 mg/day 2 month)/cured.	Thailand/2020 [12]
<i>Diaporthe miriciae</i> (MH268404)	<i>D. passifloricola</i> (NR_147595.1/98.74%) <i>D. uakeriae</i> (NR_147543.1/98.00%)	59/ female	Poorly controlled diabetes	Farmer/ Ziziphus jujuba	Soft tissue infection	Left hand	Surgery + TRB/cured	Taiwan/2021 (13)
<i>Diaporthe uakeriae</i> (OL981188)		48/ male	Renal transplant recipient	Farmer	Mycetoma	Left foot	Surgery + ITC (200 mg/day 2 m th) switched to FLC (200 mg/day 4 month). Started a new ITC treatment (Nov. 2021)/not cured	This work

^a Displayed species are those who had $\geq 99.00\%$ identity or, failing that, the two species with the highest percentages of identity.

^b FLC: fluconazole. ITC: itraconazole. VRC: voriconazole.

correlation between the cultivation of this oilseed and these cases but the expansion of the agricultural frontier may have generated a significant increase in the ecological niche of these pathogens. In turn, live weeds serve as alternative hosts for *Diaporthe* spp., providing a “green bridge” that facilitates the survival of phytopathogens between crop phases [15]. Many of these weeds have spines for adaptation, which can favor implantation by inoculation in humans.

The presented case is the second reported eumycetoma caused by *Diaporthe* spp. Using the newly published specific database, we were able to update the taxonomy of several previous cases of human mycosis caused by this fungal genus. This case highlights the need to molecularly identify fungal mycetoma agents using specific and curated databases and to evaluate their antifungal susceptibility in order to collect clinically useful data, especially if these emerging pathogens continue to increase their incidence.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Funding source

This study was supported by a CAID-orientado (Universidad Nacional del Litoral; Argentina) grant RES634/17 COD 2-2. to Guillermo Garcia-Effron.

Conflict of interest and ethical disclosure

Authors have no conflict of interest. This study was approved by the ethics committee of the Biochemistry school of the Universidad Nacional del Litoral (Res CE2021-23-C Acta 7/19). Patient has signed a written consent to publish the case report.

Acknowledgements

This study was supported by a CAID-orientado (Universidad Nacional del Litoral; Argentina) grant RES634/17 COD 2-2. to G.G.-E.

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