Medical Mycology, 2022, **60**, myac051
https://doi.org/10.1093/mmy/myac051
Original Article
Advance access publication date: 14 July 2022

Madurella mycetomatis grains within a eumycetoma lesion are clonal

Bertrand Nyuykonge [1], Emmanuel Edwar Siddig [2,3], Mickey Konings [1], Sahar Bakhiet [2], Annelies Verbon [1], Corné H.W. Klaassen [1], Ahmed Hassan Fahal [2] and Wendy W.J. van de Sande [1,*]

[1]Department of Medical Microbiology and Infectious Diseases,Erasmus MC, University Medical Centre Rotterdam, 3015 GD, Rotterdam, Netherlands
[2]Mycetoma Research Centre, University of Khartoum, JG5R+WXF, Khartoum, Sudan
[3]Faculty of medical laboratory sciences, University of Khartoum, JG5R+WXF, Khartoum, Sudan

*To whom correspondence should be addressed. Wendy van de Sande, PhD, Erasmus MC University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, P. O. Box 2040, 3000 CA Rotterdam, The Netherlands. Tel: +0031-10-7033510; E-mail: w.vandesande@erasmusmc.nl

Abstract

Eumycetoma is a neglected tropical infection of the subcutaneous tissue, characterized by tumor-like lesions and most commonly caused by the fungus *Madurella mycetomatis*. In the tissue, *M. mycetomatis* organizes itself in grains, and within a single lesion, thousands of grains can be present. The current hypothesis is that all these grains originate from a single causative agent, however, this hypothesis was never proven. Here, we used our recently developed *Mmy*STR assay, a highly discriminative typing method, to determine the genotypes of multiple grains within a single lesion. Multiple grains from surgical lesions obtained from 11 patients were isolated and genotyped using the *Mmy*STR panel. Within a single lesion, all tested grains shared the same genotype. Only in one single grain from one patient, a difference of one repeat unit in one *Mmy*STR marker was noted relative to the other grains from that patient. We conclude that within these lesions the grains originate from a single clone and that the inherent unstable nature of the microsatellite markers may lead to small genotypic differences.

Lay Abstract

In lesions of the implantation mycosis mycetoma many *Madurella mycetomatis* grains are noted. It was unknown if grains arose after implantation of a single isolate or a mixture of genetically diverse isolates. By typing the mycetoma grains we showed that all grains within a single lesion were clonal and originated from a single isolate.

Keywords: mycetoma lesions, *Mmy*STR assay, clonality, *Madurella mycetomatis*, black grains.

Introduction

Eumycetoma is a neglected tropical infection endemic in tropical and subtropical areas and is caused by several fungi. It is characterized by the formation of tumor-like lesions with multiple sinuses discharging grains.[1,2] Many fungi can cause eumycetoma, however, *Madurella mycetomatis* is the most common causative agent.[3–6] The transmission mode of eumycetoma is not clear, but it is hypothesized that the causative agent is introduced in the subcutaneous tissue via a traumatic inoculation.[7] Once inside the lesion, the fungus will form a protective structure called a fungal grain, in which the fungus itself is embedded.[8] *Madurella mycetomatis* forms black grains, which can be seen inside the tissue (Fig. 1), and in large lesions, thousands of grains can be present.[9]

The current hypothesis is that *M. mycetomatis* enters the site of infection via a small trauma, however, it is not known if only a single fungus or a mixture of fungi is introduced into the subcutaneous tissue during this trauma. The fungi that survive inside the host tissue will start to divide and grains will form. When the lesion progresses, multiple pockets with numerous grains are noted in the tissue.[10] If the grains within these pockets did arise from the introduction of a single fungus, all grains within a lesion should be genetically identical. However, if the grains within these pockets did arise from the introduction of a mixture of fungi, then mixed genotypes would be encountered in a single lesion. We recently developed a short tandem repeat (STR) assay that can be used to study the genetic variation in *M. mycetomatis* (*Mmy*STR). This assay consists of 11 STR markers specific for *M. mycetomatis* and when applied to a collection of 120 isolates assumed to be epidemiologically unrelated, resulted in a Simpson's diversity index of 0.997.[11] It can be performed on DNA isolated from cultures, but due to its high specificity and reproducibility, also on DNA isolated from *M. mycetomatis* grains embedded within clinical material.

In this study, we applied the *Mmy*STR assay to DNA isolated from grains from single lesions to determine whether the grains are clonal and derived from the introduction of a single *M. mycetomatis* strain at the site of infection or if they are of a mixed genotype and derived from multiple genetically diverse *M. mycetomatis* strains. Since individual *M. mycetomatis* isolates differ in antifungal susceptibility towards itraconazole, the current drug of choice, it is important to know if the grains in a single lesion are clonal or did arrive from multiple strains introduced into the wound simultaneously.[12]

Received: April 21, 2022. **Revised:** June 16, 2022. **Accepted:** July 12, 2022

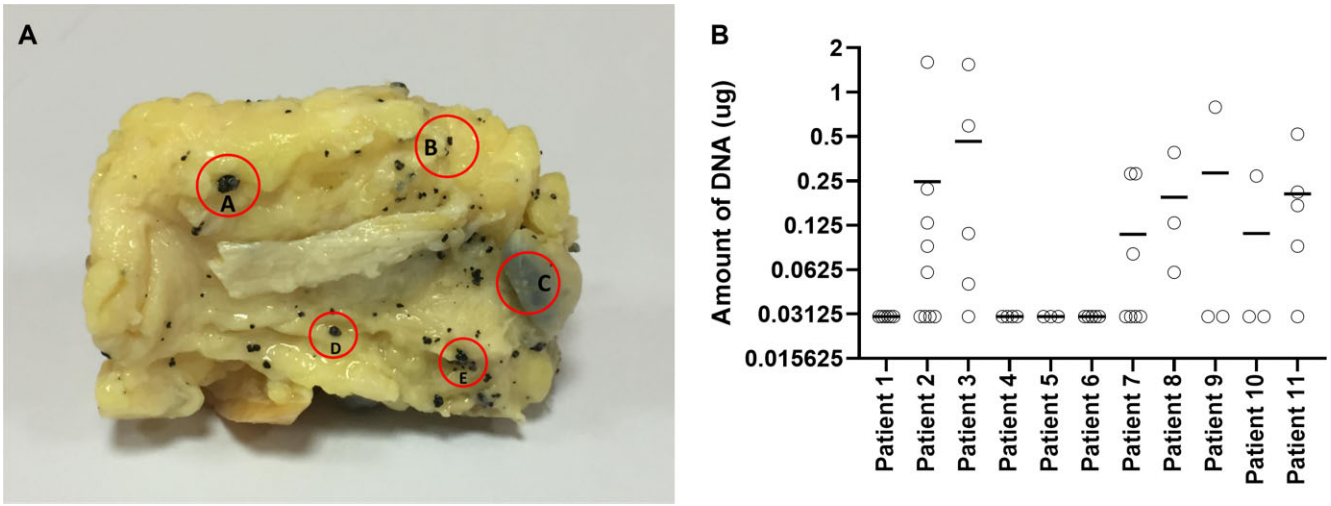

Figure 1. (**A**) Depiction of grain sampling from an encapsulated eumycetoma lesion. Grains were sampled in this case from spots A, B, C, D, and E to be used for DNA isolation and *Mmy*STR analysis. (**B**) The amount of *M. mycetomatis* DNA within the fungal grain. On average a single grain contained 0.2 ug of *M. mycetomatis* DNA with values ranging from 0.03–1.59 ug. The mean amount per patient ranged from 0.03–0.5 ug.

Table 1. Demographic data of 11 Eumycetoma patients from which grains were sampled.

Patient	Site	No. of grains	Gender	Duration of infection (years)	Location	Duration of treatment at the time of surgery (months)	Duration of treatment at the time of writing the manuscript (years)	Clinical outcome
1	Left foot	10	Male	3	Sennar	6	2	On treatment
2	Left foot	9	Male	4	Sennar	6	3	Cured
3	Left foot	5	Male	3	Sennar	6	2	On treatment
4	Right foot	7	Male	2	Sennar	6	1	On treatment
5	Left foot	5	Male	3	Sennar	6	2	On treatment
6	Left foot	5	Male	2	White Nile	6	1.5	Cured
7	Left foot	7	Male	2	White Nile	6	1	Cured
8	Left foot	3	Male	3	White Nile	6	1.5	On treatment
9	Right foot	3	Male	4	White Nile	6	3	Cured
10	Left foot	3	Male	5	White Nile	6	1.5	Cured
11	Right foot	5	Male	5	White Nile	6	3	Cured

No = Number.

Materials and methods

Patients

Eleven Sudanese patients with eumycetoma of the foot who presented at the Mycetoma Research Center, Khartoum, Sudan, were included in this study. All patients were male, five were from the Sennar State and six were from the White Nile state (Table 1). The mean duration of the disease was 3.3 years and ranged from 2 to 5 years. All patients were on itraconazole treatment, the current treatment of choice. The duration of the treatment differed per patients and ranged from 1 to 3 years, with a mean duration of treatment of 2 years (Table 1). As part of the standard mycetoma treatment, after six months of itraconazole therapy, mycetoma lesions are surgically removed. From the removed tissue, grains were sampled from different pockets within a lesion (Fig. 1(A)). This was done under sterile conditions using a different sterile surgical blade, each blade was used once to extract the grains from each pocket. On average, six grains per patient were sampled (range 3 to 11) (Table 1). Informed consent was obtained from each patient preoperatively. The study was approved by the Mycetoma Research Center, Khartoum, Sudan, ethical committee.

DNA isolation

Before DNA isolation, grains were washed with saline (0.90% w/v NaCl) twice. After individual grains were picked from the tissue, DNA from single grains was isolated using the ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research, Irvine, California, USA) according to the manufacturer's instructions with modifications. Metal beads (3 mm Ø) were used instead of ZR BashingBead™ as earlier described to ensure a high yield.[13] The DNA quality and quantity were measured using the Nanodrop (Thermo Scientific, USA).

Real-time PCR for optimization of grain DNA for MmySTR genotyping

The *Mmy*STR assay was optimized for use with 1 ng of *M. mycetomatis* DNA which generates an easy to interpret *Mmy*STR profile. Since mycetoma grains often contain a mixture of *M. mycetomatis* DNA, bacterial DNA from secondary bacterial infections[14] and human DNA, we aimed to standardize the concentration of the *M. mycetomatis* DNA within the mixed DNA sample before *Mmy*STR genotyping. For this, we used a real-time PCR targeting a *M.*

mycetomatis specific 123 bp intergenic fragment between the genes MMYC01_203 016 encoding for the transmembrane and coiled-coil domain-containing protein 4 and MMYC01_203 017 encoding for the V-type proton ATPase subunit H to approximate the concentration of *M. mycetomatis* DNA within the DNA sample. This was done by comparing Ct values of the DNA isolated from grains to those obtained using DNA isolated from a pure *M. mycetomatis* culture. Briefly, the 20 µl reaction consisted, 0.25 μm of both forward (5′-AAGAGAAGAGCAACGCGACT-3′) and reverse (5′-GTTGGCTTTCGGTGAGTGAT-3′) primers, 2 μM of SYTO^{TM} 82, and 1 ng of genomic DNA in 1x LightCyler® 480 probes master (Roche Diagnostics). Each sample had a technical replicate. The 40 cycle reaction amplification consisted of an initial denaturation step of 95°C for 5 min, followed by 40 cycles with denaturation at 95°C for 0.5 s, and annealing/extention at 60°C for 30 s. The Ct values were automatically generated and analyzed with the LightCycler software. The Ct values obtained for grain samples (Cp of sample) were compared to those of a pure culture of *M. mycetomatis* isolate MM55 (Cp of control). To obtain a DNA concentration in which ~1 ng/μl of *M. mycetomatis* DNA is present, the following formula was used $2^{(\text{Cp of control-Cp of sample})}$. In this formula, the efficiency of *M. mycetomatis* specific PCR was assumed to be 2, Cp control was the average Cp value of 1 ng/μl of DNA isolated from a *M. mycetomatis* culture and Cp of the sample was the average Ct-value of the grain sample. Finally, the normalized DNA concentrations were subsequently used for *Mmy*STR genotyping.

*Mmy*STR genotyping

The DNA samples were typed using *Mmy*STR as previously described.[15] Briefly, in each multicolor-multiplex 25 μl amplification reaction consisted of 0.5 μM concentrations of each specific primer and approximately 1 ng of *M. mycetomatis* DNA in 1x FastStart^{TM} PCR Master (Roche Diagnostics). The amplification consisted of 35 cycles with 4 min initial denaturation at 94°C, 30 s denaturation at 94°C, 30 s annealing at 55°C, 30 s extension at 72°C, and a final extension of 7 min at 72°C. The obtained PCR products were diluted 1:40 in PCR-grade water and 2 μl of the diluted product was added to 0.1 μl of GeneScan 600 LIZ (Applied Biosystems) size marker and 18 μl of HiDi formamide (Applied Biosystems). The samples were heated at 94°C for 1 min, then 4°C and injected onto an ABI 3730 XL (Applied Biosystems) following the manufacturer's instructions. The electropherograms were imported into Bionumerics software v7.6 (Applied Maths, Sint-Martens-Latem, Belgium) and analyzed using the Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA), plug-in. STR numbers were assigned relative to that of the reference sequence of strain MM55 which was included as a control.

Results

From the 11 patients included in this study, on average six (range 3–10) grains were isolated per patient, making a total of 62 grain samples from which DNA was isolated. This DNA was assumed to consist of both fungal DNA and DNA originating from the human host. In an average grain, 2.7 μg (range 0.23–9.75 μg) total DNA was present. To approximate the amount of *M. mycetomatis* DNA present, a *M. mycetomatis* specific RT-PCR was performed on the grain DNA and on DNA isolated from a cultured *M. mycetomatis* isolate. On average, each grain sample was found to have a mean of 0.2 ug of *M. mycetomatis* DNA (range 0.03–1.59 μg) (Fig. 1(B)). Low concentrations of *M. mycetomatis* DNA were observed in grains obtained from patients 1, 4, 5, and 6. More variation in *M. mycetomatis* DNA concentration was observed in grains obtained from patients 2, 3, 7, 8, 9, 10, and 11 (Fig. 1(B)). Based on these values, on average, *M. mycetomatis* DNA made up 10% of the total DNA isolated from the grain, while 90% was likely from human nature. Furthermore, there was no correlation between DNA load and patient outcome as low or high amounts of DNA were more or less the same in the 'cured' and 'on treatment' groups.

After diluting the grain samples to contain 1 ng/μl *M. mycetomatis* DNA, *Mmy*STR typing was performed. Based on the *Mmy*STR genotypes obtained, it was evident that the genotypes differed per patient. Ten different genotypes were found among the 11 patients. The genotype from grains obtained from patient 5 and patient 7 was identical (Fig. 2(A)). The genotypes encountered for the grains included in this study differed from those obtained in our previous study[11] (Fig. 2(B)).

Per patient, multiple grains obtained from a single lesion were analyzed. The genotypes obtained from grains within a lesion were identical for 10 out of 11 patients (Fig. 2(A)). Only for patient 2, a difference was noted. From the nine grains analyzed for this patient, only one grain differed in a single STR marker. That single grain had 13 repeats for marker 3C, while the other eight grains had 14 repeats for that marker. The genotypes found within a single lesion were identical for all other patients.

Discussion

Using the *Mmy*STR assay, we demonstrated that within a single lesion all grains share the same *Mmy*STR genotype indicating that a single *M. mycetomatis* isolate is introduced in the lesion during traumatic inoculation. This isolate will grow and give rise to numerous grains within the lesion. Apart from patients 5 and 7, who were infected with a *M. mycetomatis* isolate sharing the same *Mmy*STR genotype, all the other genotypes appeared genetically distinct, supporting our previous observation that *M. mycetomatis* is genetically divergent.[15] Furthermore, these new genotypes were novel as they were not observed or identified previously.[15] It is unclear why patients 5 and 7 were infected with genetically similar *M. mycetomatis* isolates. These patients originated from two different states, but these two states share a common boundary. It could be that they contracted the infection at a common site, however, since the patients were lost to follow up, we could not investigate this further.

From the nine grains analyzed for patient 2, one grain differed in a single STR marker by one repeat, suggesting that in massive lesions instability events can take place. Either during the four years the patient had this lesion or during therapy, this micro-evolution event in STR marker 3C occurred. Variation in the number of tandem repeats can occur by strand-slippage of the DNA polymerase during replication or recombination.[16,17] Micro-evolution within fungal infections has been reported before[18,19] and is often at random locations within

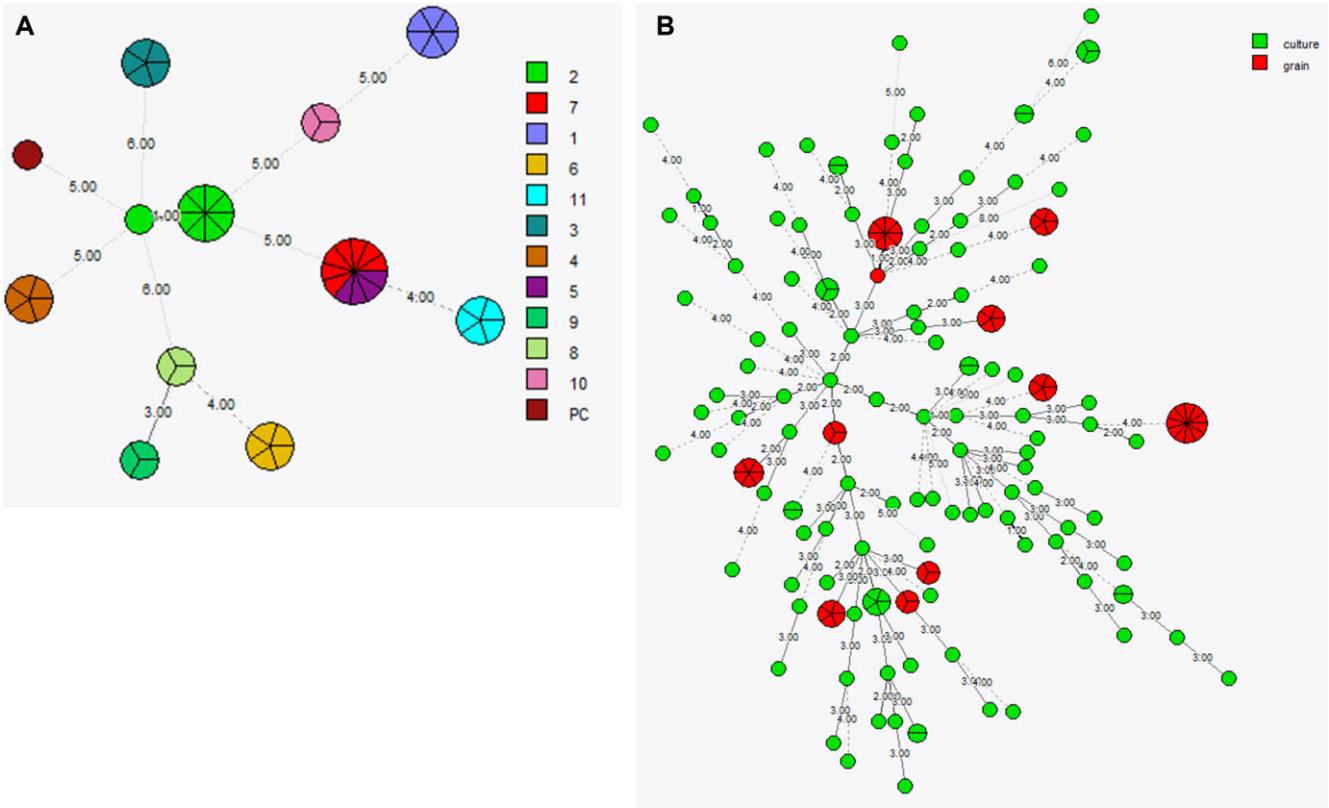

Figure 2. Minimum spanning tree (MST) showing genetic diversity of grains obtained from 11 eumycetoma patients. **(A)** No evidence of mixed genotypes within a single lesion of 11 eumycetoma caused *M. mycetomatis*. Each circle represents a genotype and the size is proportional to the number of isolates within the genotype. Numbers on connecting lines represent the number of different markers between genotypes. Each color represents a particular patient depicted by a number, while PC is the reference strain genotype (MM55). (**B**) All genotypes obtained from 11 patients (red) were novel as none clustered with previously described genotypes (green). Here, each circle represents a genotype and the size is directly proportional to the number of isolates of that genotype.

the genome. However, mutation rates in tandem repeats are usually a factor of 10 or more frequent than point mutations elsewhere in the genome.[17] For *Aspergillus fumigatus*, a similar short tandem repeat assay (STR*Af*) was already developed in 2005. In this STR*Af* assay, similar micro-evolutionary events were also noted in two STR markers. They occurred in a single marker after five generations for two isolates and 36 generations for the third isolate. The other seven markers remained stable within 473 generations.[20] Similar to the micro-evolutionary event we noted in our *Mmy*STR assay, all three micro-evolutionary observed with the STR*Af* assay occurred in a trinucleotide repeat marker with a relatively high copy number. Both features have been known to affect STR stability. Regarding unit-length, the effect of the length of the repeat is not always clear. Some studies demonstrate that a shorter unit length leads to more instability,[21] while other studies demonstrate that a higher unit length leads to more instability.[20,22] It is striking that for both *A. fumigatus* and *M. mycetomatis* the trinucleotide repeats were involved in micro-evolutionary events. With regards to the length of the repeat for *A. fumigatus*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Arabidopsis thaliana* , and *Homo sapiens* it was demonstrated that instability occurs more often if the number of repeats is higher.[20,23–25] However, for *M. mycetomatis*, marker 3C had 14 repeats before this micro-evolutionary event showing which is shorter compared to the unstable trinucleotide markers in the STRAf assay and also denotes a deletion contrary to STRAf[20] but similar to what was observed in other studies.[26]

Since *Mmy*STR determines genetic variation by analyzing 11 selected STR markers covering only a tiny fraction of the fungal genome, we will not detect all genetic variation occurring between the individual grains. The agreement between *Mmy*STR and Whole Genome Sequencing (WGS) has not been studied for *M. mycetomatis* due to the lack of available genomes. At this moment, only five *M. mycetomatis* genomes are available in the NCBI Genbank.[27,28] However, for other fungal species such as *Candida auris*, a comparison between STR typing and WGS has been performed and demonstrated that the *C. auris* STR assay consisting of 23 STR markers could differentiate *C. auris* isolates which differed by $\geq$ 30 single nucleotide polymorphisms (SNPs), per genome. However, when strains only differed by 20 SNPs or less, they would be not be differentiated by the STR assay.[29] This indicates that STR assays could be comparable to WGS.

The treatment of eumycetoma is characterized by recurrences,[30,31] and it is not clear what the role of antimicrobial resistance is at the start or during treatment. The finding that mycetoma lesions are formed after the introduction of a single *M. mycetomatis* strain suggests that at the start of treatment all individual grains have an equal susceptibility profile for the drug given. This indicates that susceptibility testing can be performed on a single isolate and there is no need to test

multiple isolates from the same lesion. A limitation of the current study is that we sacrified the complete grain for DNA isolation which prevented us to grow the isolate as well and perform *in vitro* susceptibility testing. We therefore could not verify that all grains within the lesion would have indeed an equal susceptibility profile. We did demonstrate that micro-evolution within the lesion can occur. For *C. albicans* it has been demonstrated that this micro-evolution can also occur in the azole target gene ERG11 and in the transcriptional activator of the multidrug efflux transporter CDR.[19] It is, therefore, important to note that micro-evolution can occur during the infection and that the *Mmy*STR assay will not pick up micro-evolutionary events outside the 11 selected STR markers. To identify these events, sequencing of the azole target gene and genes associated with azole resistance in other fungi can be performed by classical Sanger sequencing or by WGS and the rise of resistance can be determined by *in vitro* susceptibility testing.

Based on the data presented here, we can conclude that a eumycetoma lesion originates from the introduction of a single *M. mycetomatis* strain in the subcutaneous tissue and that all grains will have the same susceptibility for antifungal therapy. Micro-evolution can occur during the long time before and during treatment and further research is warranted whether this will result in selection of resistance against antifungal drugs. Furthermore, it will be interesting to sample and analyse grains obtained from lesions in different parts of the body in the future.

Author contributions

BN: formal analysis, data collection, validation, visualization, writing–original draft preparation and review/editing. **EES, SB,** and **AHF** patient sampling, original draft preparation and review/editing. **MK**: writing and review/editing. **CHW:** Formal analysis, supervision, validation, visualization, original, review/editing. **AV**: supervision, validation, visualization, original draft preparation, and review/editing. **WWJvdS**: conceptualization, project administration, formal analysis, supervision, validation, visualization, original, draft preparation, and review/editing.

Declaration of interest

None.

References

1. Zijlstra EE, van de Sande WWJ, Welsh O et al. Mycetoma: a unique neglected tropical disease. *Lancet Infect Dis*. 2016; 16 (1): 100–112.
2. van de Sande WW, Maghoub eS, Fahal AH et al. The mycetoma knowledge gap: identification of research priorities. *PLoS Negl Trop Dis*. 2014; 8 (3): e2667.
3. Ahmed SA, van de Sande WW, Stevens DA et al. Revision of agents of black-grain eumycetoma in the order Pleosporales. *Persoonia*. 2014; 33 (1): 141–154.
4. de Hoog GS, van Diepeningen AD, Mahgoub e-S, van de Sande WW. New species of Madurella, causative agents of black-grain mycetoma. *J Clin Microbiol*. 2012; 50 (3): 988–994.
5. Ahmed SA, van den Ende BH, Fahal AH, van de Sande WW, de Hoog GS. Rapid identification of black grain eumycetoma causative agents using rolling circle amplification. *PLoS Negl Trop Dis*. 2014; 8 (12): e3368.
6. Nyuykonge B, Klaassen CHW, Zandijk WHA et al. Diagnostic implications of mycetoma derived from Madurella pseudomycetomatis isolates from Mexico. *J Eur Acad Dermatol Venereol*. 2020; 34 (8): 1828–1834.
7. de Hoog GS, Ahmed SA, Najafzadeh MJ et al. Phylogenetic findings suggest possible new habitat and routes of infection of human eumyctoma. *PLoS Negl Trop Dis*. 2013; 7 (5): e2229.
8. Sheehan G, Konings M, Lim W et al. Proteomic analysis of the processes leading to Madurella mycetomatis grain formation in Galleria mellonella larvae. *PLoS Negl Trop Dis*. 2020; 14 (4): e0008190.
9. Ibrahim AI, El Hassan AM, Fahal A, van de Sande WW. A histopathological exploration of the Madurella mycetomatis grain. *PLoS One*. 2013; 8 (3): e57774.
10. Fahal AH, Suliman SH, Mycetoma Hay R.: The spectrum of clinical presentation. *Trop Med Infect Dis*. 2018; 3 (3): 97.
11. Balajee SA, de Valk HA, Lasker BA, Meis JF, Klaassen CH. Utility of a microsatellite assay for identifying clonally related outbreak isolates of Aspergillus fumigatus. *J Microbiol Methods*. 2008; 73 (3): 252–256.
12. van Belkum A, Fahal AH, van de Sande WW. *In vitro* susceptibility of *Madurella mycetomatis* to posaconazole and terbinafine. *Antimicrob Agents Chemother*. 2011; 55 (4): 1771–1773.
13. Ahmed SA, van de Sande WW, Desnos-Ollivier M et al. Application of isothermal amplification techniques for identification of *Madurella mycetomatis*, the prevalent agent of human Mycetoma. *J Clin Microbiol*. 2015; 53 (10): 3280–3285.
14. Ahmed AO, Abugroun ES. Unexpected high prevalence of secondary bacterial infection in patients with mycetoma. *J Clin Microbiol*. 1998; 36 (3): 850–851.
15. Nyuykonge B, Eadie K, Zandijk WHA et al. A short-tandem-repeat assay (*Mmy*STR) for studying genetic variation in *Madurella mycetomatis*. *J Clin Microbiol*. 2021; 59 (3): e02331–20
16. Ananda G, Walsh E, Jacob KD et al. Distinct mutational behaviors differentiate short tandem repeats from microsatellites in the human genome. *Genome Biol Evol*. 2013; 5 (3): 606–620.
17. Gemayel R, Vinces MD, Legendre M, Verstrepen KJ. Variable tandem repeats accelerate evolution of coding and regulatory sequences. *Annu Rev Genet*. 2010; 44: 445–477.
18. Pais P, Galocha M, Viana R et al. Microevolution of the pathogenic yeasts. *Microb Cell*. 2019; 6 (3): 142–159.
19. Siikala E, Rautemaa R, Richardson M et al. Persistent *Candida albicans* colonization and molecular mechanisms of azole resistance in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients. *J Antimicrob Chemother*. 2010; 65 (12): 2505–2513.
20. de Groot T, Meis JF. Microsatellite stability in STR analysis aspergillus fumigatus depends on number of repeat units. *Front Cell Infect Microbiol*. 2019; 9: 82.
21. Schug MD, Hutter CM, Wetterstrand KA et al. The mutation rates of di-, tri-, and tetranucleotide repeats in *Drosophila melanogaster*. *Mol Biol Evol*. 1998; 15 (12): 1751–1760.
22. Payseur BA, Jing P, Haasl RJ. A genomic portrait of human microsatellite variation. *Mol Biol Evol*. 2011; 28 (1): 303–312.
23. Flores C, Engels W. Microsatellite instability in Drosophila spellchecker1 (MutS homolog) mutants. *Proc Natl Acad Sci U S A*. 1999; 96 (6): 2964–2969.
24. Sia EA, Kokoska RJ, Dominska M, Greenwell P, Petes TD. Microsatellite instability in yeast: dependence on repeat unit size and DNA mismatch repair genes. *Mol Cell Biol*. 1997; 17 (5): 2851–2858.
25. Wierdl M, Dominska M, Petes TD. Microsatellite instability in yeast: dependence on the length of the microsatellite. *Genetics*. 1997; 146 (3): 769–779.
26. Vogler AJ, Keys C, Nemoto Y et al. Effect of repeat copy number on variable-number tandem repeat mutations in Escherichia coli O157:H7. *J Bacteriol*. 2006; 188 (12): 4253–4263.
27. Khidir ES, Ahmed A, Fahal AH, Ibrahim AA. Draft genome sequences of three clinical isolates of Madurella mycetomatis, the

major cause of black-grain Mycetoma. *Microbiol Resour Announc*. 2020; 9 (16): e01533–e01619.
28. Smit S, Derks MF, Bervoets S et al. Genome sequence of *Madurella mycetomatis* mm55, isolated from a human mycetoma case in Sudan. *Genome Announc*. 2016; 4 (3): e00418–e00516.
29. de Groot T, Puts Y, Berrio I, Chowdhary A, Meis JF. Development of candida auris short tandem repeat typing and its application to a global collection of isolates. *mBio*. 2020; 11 (1): e02971–19.
30. Borjian Boroujeni Z, Hashemi SJ, Daie Ghazvini R et al. Recurrent eumycetoma caused by novel species. *Med Mycol Case Rep*. 2019; 26: 13–15.
31. Suleiman SH, Wadaella eS, Fahal AH. The surgical treatment of Mycetoma. *PLoS Negl Trop Dis*. 2016; 10 (6): e0004690.