International Journal of Infectious Diseases 124 (2022) 224–226

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

Staphylococcus aureus causing primary foot botryomycosis mimicking actinomycetoma: a case report from Sudan

Emmanuel Edwar Siddig[1,2,3], Bertrand Nyuykonge[2], Osama EL Hadi Bakheit[1], Omnia Babekir Hassan[1], Eiman Siddig Ahmed[1], Asma Adam Osman[1], Sahar Mubarak Bakhiet[4], Wendy WJ van de Sande[2,#,*], Ahmed Hassan Fahal[1,#]

[1] *The Mycetoma Research Center, University of Khartoum, Khartoum, Sudan*
[2] *Erasmus MC, University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, Rotterdam, The Netherlands*
[3] *Unit of Basic Medical Sciences, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan*
[4] *Department of Molecular Biology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan*

ARTICLE INFO

Article history:
Received 26 July 2022
Revised 3 October 2022
Accepted 6 October 2022

Keywords:
Botryomycosis
Staphylococcus aureus
Mycetoma
Actinomycetoma
Diagnosis
Sudan

ABSTRACT

Objectives: Botryomycosis is a rare chronic granulomatous inflammatory disease of bacterial origin. Two forms of the disease exist; the cutaneous and the visceral form. The subcutaneous form mimics actinomycetoma clinically and histologically; however, the treatment is different. In this communication, we report on a Sudanese male patient who presented with foot botryomycosis.
Design: Case report.
Results: The patient was initially diagnosed with actinomycetoma by the presence of *Streptomyces somaliensis* like-grains in the histological slides. The patient was treated with a combination of co-trimoxazole and amikacin sulfate and shifted after 1 year to co-trimoxazole, amoxicillin, and clavulanic acid. Despite treatment, the infection progressed, and the bone was invaded. The infected limb was amputated. The histopathological report of the surgical biopsy showed gram-positive cocci inside the grain. The 16S sequence identified these cocci as *Staphylococcus aureus.*
Conclusion: This is the first reported botryomycosis case from Sudan, and it highlights why molecular identification is vital in diagnosis.

Introduction

Botryomycosis and actinomycetoma are chronic granulomatous subcutaneous inflammatory diseases caused by bacteria; however, the etiology differs between the two diseases (Bonifaz and Carrasco, 1996; Padilla-Desgarennes et al., 2012; Yendo et al., 2021). Botryomycosis is most often caused by commensal bacteria such as the gram-positive coccus *Staphylococcus aureus* (Padilla-Desgarennes et al., 2012), actinomycetoma is most often caused by actinomycetes such as *Nocardia brasiliensis, Actinomadura madurae,* and *Streptomyces somaliensis* (Siddig et al., 2021). It is difficult to diagnose botryomycosis on clinical examination alone or with the present diagnostic tools. Hence, many cases may be missed as actinomycetoma in mycetoma-endemic regions. To our knowledge, the present case (Figure 1a) is the first verified case of cutaneous botryomycosis caused by *S. aureus* in Sudan that mimics actinomycetoma.

Case presentation

A 25-year-old male from the White Nile State, Sudan, presented to the Mycetoma Research Centre (MRC), Khartoum, Sudan, in June 2014 with a painless swelling on the right foot. It measured 3.4 × 3.8 cm in diameter and was located on the sole. His condition started 1 year before with a small painless swelling that did not interfere with his daily activities. Hence he ignored it. The swelling gradually increased in size to involve the dorsal aspect of the foot. Multiple secondary swellings developed with numerous sinuses and sero-purulent discharge containing yellow to white grain-like structures. The patient had no history of local trauma, family history of mycetoma, or treatment of the foot

Abbreviation: H&E, Hematoxylin and Eosin.

* Corresponding author: Wendy WJ van de Sande, Erasmus MC, University Medical Center Rotterdam, Department of Medical Microbiology and Infectious Diseases, Rotterdam, The Netherlands.
E-mail address: w.vandesande@erasmusmc.nl (W.W. van de Sande).

# Contributed equally.

https://doi.org/10.1016/j.ijid.2022.10.010

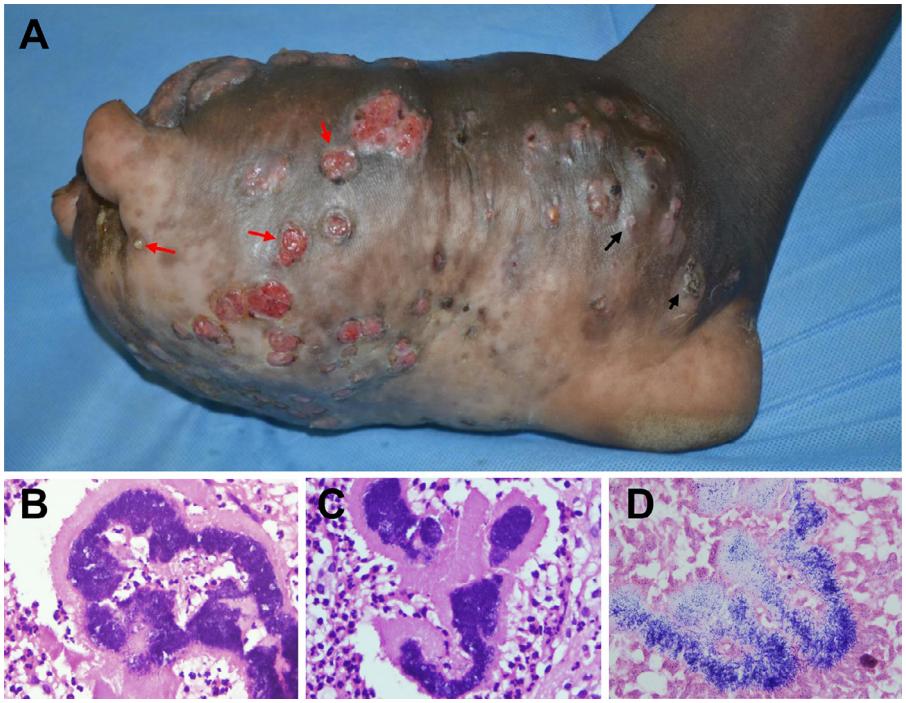

Figure 1. (a) Photography showing the massive foot botryomycosis with multiple active (red arrows) and healed (black arrows) sinuses. (b) The 2014 microphotography of a histological section showing a grain surrounded by inflammatory cells. The grain is irregular in shape, stained blue with (H&E) and diagnosed as *Streptomyces somaliensis*. The *S. somaliensis* grains usually stain pink in H&E staining (H&E 400X). (c) The 2018 histological section showing a grain irregular in shape, stained blue with H&E with an abundant extracellular matrix. The grain is surrounded by granulation tissue with marked histiocytic and mixed inflammatory cellular infiltrates. A Splendore-Hoeppli reaction is noted at the section periphery and stained light red. (H&E 400X). (d) A Gram-stained histological section showing gram-positive cocci inside the grain and the surrounding tissue. (Gram 400X).
Abbreviation: H&E, hematoxylin and eosin.

swelling. On clinical examination, he looked well, not pale, jaundiced, or cyanosed. His systemic examinations were within normal. No regional lymphadenopathy was noted, and his full blood count was within normal, including hemoglobin of 14.7 g/dl, a white blood cell count of 6600, and a platelet count of 303 $\times$ 10^9/l. An ultrasonographic scan of the lesion revealed multiple pockets containing fluid and fine echogenic grains suggestive of actinomycetoma. Right foot x-rays in anteroposterior and lateral views showed soft tissue mass with intact bones. A deep surgical biopsy was taken to obtain grains for histopathology and culture. The hematoxylin and eosin (H&E) stained histopathological sections showed *S. somaliensis*-like-grains with type I and II tissue reactions (Fahal et al., 1995) (Figure 1b). The grains were isolated and incubated on yeast extract agar for 6 weeks, but no growth suggestive of actinomycetes was observed. According to the MRC actinomycetoma treatment protocol, the patient was started on 960 mg co-trimoxazole twice daily for 5 weeks, 15 mg/kg amikacin twice daily for 3 weeks, and 5 mg folic acid once daily for 5 weeks in the form of cycles. He took one cycle and was lost for follow-up for 3 years.

In 2017, the patient returned to MRC with massive swelling involving the complete right foot and the ankle joint region. He was unable to walk and was using walking aids. Local examination revealed a painful mass of 24 $\times$ 10 cm in size involving the sole and dorsal aspects of the foot. There were multiple active and healed sinuses and discharge (Figure 1a). The swelling became offensive in odor. Multiple sinuses discharging pale to yellow grains and pus were noted. Systemic examinations were within normal. He restarted the MRC actinomycetoma treatment protocol. On February 10, 2018, the patient presented with a massive painful lesion. The right foot x-ray showed massive destruction and cavitation of the whole foot bones. On December 16, 2019, the affected foot was amputated. The histopathological slides from the amputated foot showed scattered grains different from actinomycetoma grains. In the sections, the bacteria were surrounded by a zone of a large eosinophilic matrix with club-like projections. A Splendore-Hoeppli phenomenon was noted. The grains had no cracks, and the bacteria stained dark blue with H&E (Figure 1c). These differed from typical *S. somaliensis* grains, which are large with multiple cracks and have the eosin color (Siddig et al., 2019, 2021). The bacterial grains were also different from *A. madurae* and *Actinomadura pelletieri* grains. These species tend to form thicker grains without cracking and only a small Splendore-Hoeppli reaction at the periphery. Furthermore, grains of *A. pelletieri* are red, while the grains observed in this patient are white to yellow in color. An additional Gram stain was done, which demonstrated the presence of gram-positive cocci aggregated within the grains and distributed in the adjacent tissues (Figure 1d). To identify the causative agent, DNA was extracted, and the internal transcribed spacer region and 16S region were amplified and sequenced (Nyuykonge et al., 2020). Only the 16S polymerase chain reaction was positive, and the consensus sequence was uploaded onto Genbank with accession number (OM478580). The sequence was 100% identical to *S. aureus* (MZ951147.1).

The patient was seen on October 5, 2020, in good shape with no evidence of recurrence. The tumor-like mass had disappeared completely, and following ultrasound examination, no evidence of disease was present. This lesional ultrasound examination was repeated three times at 3-month intervals, and there was still no evidence of recurrence.

Discussion

Since botryomycosis and actinomycetoma resemble each other clinically and radiologically, it is not surprising that in mycetoma-endemic regions, botryomycosis patients can be misdiagnosed as

actinomycetoma. Our patient was initially misdiagnosed based on the presence of grains in the exudate, ultrasound examination, and histopathology. Only the second histopathologist viewing the histopathology sections after amputation noted that the grains were different from those seen in *S. somaliensis*. Their color was violet in the H&E section instead of the pink color normally observed for *S. somaliensis* (Siddig et al., 2019, 2021). Furthermore, no grain cracking was noticed, and the Splendore-Hoeppli phenomenon was more marked and intense compared to the usual *Streptomyces* grains. This was not observed initially, indicating that the experience of the histopathologist is vital for data interpretation and proper diagnosis. No growth indicative of *S. somaliensis* was observed when the grains were inoculated on agar. In mycetoma lesions, secondary bacterial infections are often present, and *S. aureus* is the most common cause of these infections (Ahmed et al., 1998). Therefore the observed *S. aureus* growth might have been considered a contaminant. In the reported patient, sequencing the 16S region revealed *S. aureus* as the botryomycosis causative agent, but since it can also be present as a secondary side infection in mycetoma cases, molecular detection of *S. aureus* alone is not enough to establish the diagnosis of botryomycosis. Hence, Gram staining of the histological section helps to confirm the diagnosis.

Botryomycosis usually responds to a combination of antibiotics that include co-trimoxazole, rifampin, minocycline, erythromycin, and cephalosporins (Bonifaz and Carrasco, 1996; Shimagaki et al., 2020; Yendo et al., 2021). This combination is similar to that used to treat the different actinomycetoma causative organisms (Siddig et al., 2021; Welsh et al., 2014). However, the reported patient did not respond to the antibiotic therapy prescribed. Since the drug sensitivities were not determined in this patient, it will be difficult to know the drug sensitivity of the causative organism. However, in general, in Sudan, *S. aureus* is susceptible to co-trimoxazole and amikacin sulfate and often resistant to amoxicillin (Hamid et al., 2020). As in many other countries, in Sudan, there is extensive usage of antibiotics without prescription, which leads to increased levels of resistance (Ahmed, 2020). The three patients with botryomycosis caused by *S. aureus* reported by Sirka et al. in 2019 were treated with a different regimen (Sirka et al., 2019). Two of them were treated orally with 100 mg doxycycline twice daily. The third patient was treated with 500 mg ciprofloxacin twice daily. The patient treated with ciprofloxacin showed complete resolution after 8 weeks. One patient treated with doxycycline had a complete resolution after 4 weeks. The second patient treated with doxycycline had 60-80% resolution in 20 days (Sirka et al., 2019).

In conclusion, we report on the first foot botryomycosis caused by *S. aureus* from Sudan. Clinicians and care providers should be aware of botryomycosis as a clinical entity. It can be confused with actinomycetoma clinically, and the diagnosis needs experience as accurate diagnosis is key in patient management.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics approval and patient consent

Ethical approval was obtained from the MRC IRB. The patient gave written consent.

Authors' contributions

Emmanuel Edwar Siddig (EES), Wendy WJ. van de Sande (WWJ) and Ahmed Hassan Fahal (AHF) conceived the study; EES, Osama EL Hadi Bakheit (OEB), Omnia Babekir Hassan (OBH), Eiman Siddig Ahmed (ESA), Asma Adam Osman (AAO), AHF collect the data; EES, Bertrand Nyuykonge (BN), ESA, OEB, WWJ, and AHF were responsible for analysis and interpretation of data, and revision. All authors approved the final draft.

Consent for publication

Not applicable.

Availability of data and materials

The data is available here.

Declaration of competing interests

The authors have no competing interests to declare.

References

Ahmed AOA, Abugroun ESAM, Fahal AH, Zijlstra EE, van Belkum A, Verbrugh HA. Unexpected high prevalence of secondary bacterial infection in patients with mycetoma. J Clin Microbiol 1998;36:850–1.
Ahmed OB. Prevalence of methicillin-resistant Staphylococcus aureus and classical enterotoxin genes among Sudanese food handlers. Cureus 2020;12:e12289.
Bonifaz A, Carrasco E. Botryomycosis. Int J Dermatol 1996;35:381–8.
Fahal AH, el Toum EA, el Hassan AM, Mahgoub ES, Gumaa SA. The host tissue reaction to Madurella mycetomatis: new classification. J Med Vet Mycol 1995;33:15–17.
Hamid MH, Arbab AH, Yousef BA. Bacteriological profile and antibiotic susceptibility of diabetic foot infections at Ribat University Hospital; a retrospective study from Sudan. J Diabetes Metab Disord 2020;19:1397–406.
Nyuykonge B, Klaassen CHW, Zandijk WHA, de Hoog GS, Ahmed SA, Desnos-Ollivier M, et al. Diagnostic Implications of Mycetoma derived from Madurella pseudomycetomatis isolates from Mexico. J Eur Acad Dermatol Venereol 2020;34:1828–34.
Padilla-Desgarennes C, Vázquez-González D, Bonifaz A. Botryomycosis. Clin Dermatol 2012;30:397–402.
Shimagaki H, Yuki A, Kimura K, Yuki D, Fujikawa H, Shimizu N, et al. Case of cutaneous botryomycosis in an 8-year-old immunocompetent boy with a review of the published work. J Dermatol 2020;47:542–5.
Siddig EE, Mhmoud NA, Bakhiet SM, Abdallah OB, Mekki SO, El Dawi NI, et al. The accuracy of histopathological and cytopathological techniques in the identification of the mycetoma causative agents. PLoS Negl Trop Dis 2019;13.
Siddig EE, van de Sande WWJ, Fahal AH. Actinomycetoma laboratory-based diagnosis: a mini-review. Trans R Soc Trop Med Hyg 2021;115:355–63.
Sirka CS, Dash G, Pradhan S, Naik S, Rout AN, Sahu K. Cutaneous botryomycosis in immunocompetent patients: a case series. Indian Dermatol Online J 2019;10:311–15.
Welsh O, Al-Abdely HM, Salinas-Carmona MC, Fahal AH. Mycetoma medical therapy. PLoS Negl Trop Dis 2014;8:e3218.
Yendo TM, Valente NS, Nico MMS. Botryomycosis caused by Staphylococcus warneri: the first case reported. Int J Dermatol 2021;60:509–11.