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Case Report

# A Rare Opportunistic Skin Infection Caused by *Exserohilum Rostratum* in a Young Boy with Acute Leukemia in Italy and Literature Review

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## Abstract

**Background**: *Exserohilum* spp. are environmental molds that may rarely cause skin infections especially in immunocompromised children. The present report describes the first case of cutaneous phaeohyphomycosis in Italy, caused by *Exserohilum rostratum* in a child undergoing treatment for leukemia, and a review of the literature of pediatric infections. **Case Description**: The patient presented with a supramalleolar main granulomatous lesion, which later spread to the other limb. The patient underwent a routine hospital examination, and a biopsy of the lesion was positive for the fungus *Exserohilum rostratum*, which was first identified by mass spectrometry and then confirmed by NGS on the Illumina platform. **Conclusion**: From 1975 to 2012, 48 *Exserohilum rostratum* infections have been reported in the literature, including 17 from immunocompromised pediatric patients. Identification of this rare fungus is critical for appropriate therapeutic approaches. NGS currently represents an excellent technology for the identification of rare fungal pathogens as in our case. Therapy with amphotericin B alone or in combination with azoles has been critical for the resolution of skin lesions.

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**Keywords**: *Exserohilum Rostratum*; Child; Skin Infection; NGS; Acute T Lymphoblastic Leukemia

## Introduction

Phaeohyphomycosis is a group of fungal infections caused by dark-pigmented, melanin-containing dematiaceous fungi. The main etiological agents of phaeohyphomycosis are the species Bipolaris, Exophiala, Curvularia, Chaetomium, Phoma, *Exserohilum*, and Wangiella [1]. Their infections are implicated in variety of clinical presentations, ranging from superficial to deep-seated infections [2], including skin and soft tissue infections, rhinosinusitis, lung, corneal, disseminated, and central nervous system diseases [3,4]. The genus *Exserohilum* includes approximately 35 species and is a common saprophytic fungus of plants in warm, humid climates. Rarely can be pathogenic to humans, especially in tropical and subtropical regions [5]. So far, three human pathogenic species have been isolated, both in immunocompromised and immunocompetent hosts belonging to the *Exserohilum rostratum*, *Exserohilum* longi*rostratum* and *Exserohilum* mcginnisii [6,7]. The infection is commonly acquired from minor trauma or inhalation and the fungus is found in soil, organic material, plants, and air, but is not transmitted from person to person.

The first *Exserohilum rostratum* outbreak was reported in 2012 by the Centers for Disease Control and Prevention (CDC) in patients who had received injections, primarily epidural, of methylprednisolone acetate (MPA) produced by the New England Compounding Center in Framingham, Massachusetts [6].

Here, we describe the first Italian case of *Exserohilum rostratum* infection in a 15-year-old boy with acute lymphoblatic leukemia, and then we provide a summary of *Exserohilum rostratum* infections in immunocompromised children affected by hematological diseases.

## Case Description

### Patient Information

A 15-year-old boy, originally from Senegal, arrived at the Ravenna Emergency Department on 23 April 2023 (Day 0) with malaise, petechiae, and ulcers. The boy had arrived in Italy for a family reunification with his father at the beginning of 2023, already suffering numerous minor traumas to his legs and skin lesions, resulting from contact with the ground and branches during his daily activities in Senegal. Upon admission, an empirical antimicrobial therapy was started using a third-generation cephalosporin (Ceftriaxone 2g/day for two weeks), due to the presence of fever [8]. After the diagnosis of acute leukemia, he was initiated on prednisone therapy (Deltacortene 37 mg twice daily for three weeks). The chemotherapy regimen was performed according to the International Collaborative Treatment Protocol for Children and Adolescents with Acute Lymphoblastic Leukemia [9], with Vincristine and Daunorubicin (day +8) and the addition of Cyclophosphamide (day +10). A lumbar puncture with Methotrexate infusion was also performed (day +12), then repeated therapy with Vincristine and Daunorubicin (day +15) and Pegaspargase was administered at day +19. A second cycle of this protocol was started on May 16 (day +20). From June 15 (day +48), cytarabine was started.

### Clinical Findings

On May 5th (day +9), a few days after starting steroid therapy, the boy developed a single nodular, hard, painless skin lesion in the supramalleolar region of his right leg (Figure 1A). The nodule was 5 cm in diameter and showed no typical signs of skin infection: no erythema, no pain, no warmth, and no itching. An ultrasound examination of the skin and soft tissue revealed thickening of the subcutaneous adipose tissue over a longitudinal extent of 4.5 cm and a thickness of 0.7 cm. There was no evidence of localized fluid collections or fluid imbibition of the adipose tissue. On the same day, an infectious disease consultation was requested, during the examination, the patient showed no clinical or laboratory signs of an ongoing infection, testing negative in molecular and serological research on HIV, CMV, HCV, HBV, HHV6-7, EBV, VZV, Rubella, Toxoplasma, Measles and Mycobacteria, but he had been neutropenic for a week. Due to the absence of systemic and local signs of infection, no new anti-infective therapy was initiated. The lesion rapidly progressed to a cutaneous ulcer. On May 11th (Day +15), a biopsy was performed for histological and cultural examination. While awaiting the biopsy results, the boy developed three new skin lesions: one on the back of his right hand, one on the distal phalanx of the fourth finger of his left hand, and one on the back of his left foot. All the lesions presented as hard, painless nodules with a granular consistency, without skin erythema or warmth. Despite the boy being afebrile and showing no systemic signs of infection, the pediatricians and the infectious disease specialist agreed to initiate antifungal prophylaxis with Liposomal Amphotericin B (AMB). On May 17th (day +21), based on histological results, antifungal therapy with 250 mg/day of AMB was initiated. The histological examination revealed atypical lymphoid infiltrates consistent with the localization of acute T lymphoblastic leukemia and the widespread presence of fungal forms in interstitial dermal and intravascular locations.

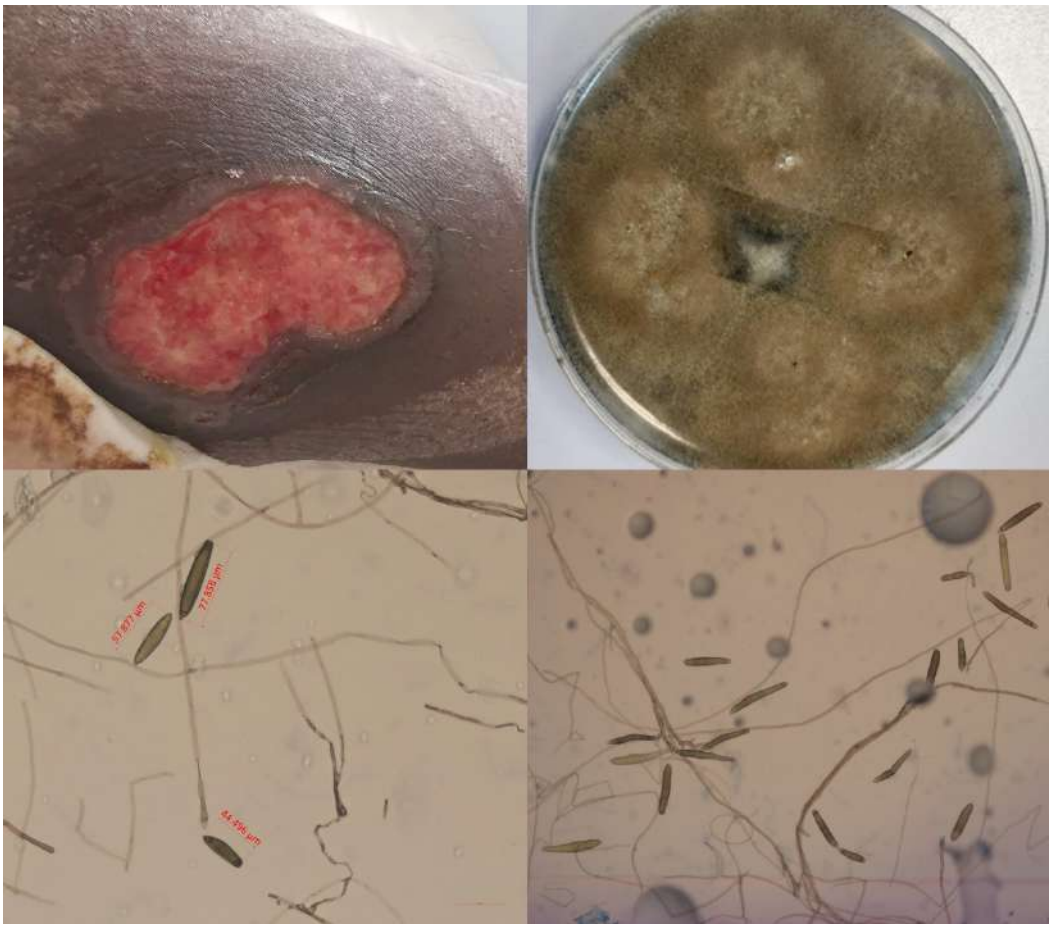

**Figure 1**: (A) excised supramalleolar lesion originating from the right leg, from which the biopsy was performed (B) dark and cottony macroscopic appearance of the *Exserohilum rostratum* colony on SGC2 after seven days of incubation at 30°C, (C-D) optical microscope images of *Exserohilum rostratum* sympodial conidiophore with cylindrical-ellipsoidal conidia (44-77 µm) and dark brown hyphae (200X).

**Diagnostic Assessment**

The microbiological culture yielded a dematiaceous fungus after 7 days (day +22) of incubation at 30°C on Sabouraud Dextrose Agar (SGC2, Biomerieux). Initially, the fungus appeared white, but 2-3 days (day +25) later, it acquired a brown/black pigmentation on both the front and the back (Figure 1B). The fungus was initially identified using Mass Spectroscopy (MALDI-TOF MS, MS Prime Biomerieux) as *Exserohilum rostratum*, and the identification was further confirmed by Next-Generation Sequencing (NGS) of the Internal Transcribed Spacer (ITS1) on the Illumina MiSeq platform. The reads were analyzed on BaseSpace Sequence Hub throught the pipeline 16S Metagenomics with the UNITE Fungal ITS Database v7.2. The sequence was deposited in the NCBI SRA (Sequence Read Archive) database under the accession number PRJNA990688. Following several subcultures on Potato Dextrose Agar (PDA, Biomerieux) at 30°C, we observed the production of conidia (Figure 1C-D), enabling us to assess the in vitro susceptibility testing by using broth microdilution following EUCAST definitive document E.DEF 9.4 [10] filamentous fungi. The Minimum Inhibitory Concentrations (MICs) were as follows: AMB ≤0.12mg/L, Isavuconazole 1mg/L, Itraconazole ≤0.015mg/L, Posaconazole 0.12mg/L, Voriconazole 0.06 mg/L. These MIC values were in the range of those reported for *Exserohilum rostratum* by Chowdhary et al. [2].

**Therapeutic Intervention**

Considering the clinical status and the laboratory results, the supramalleolar lesion has been removed surgically. To facilitate the patient's discharge home, after fifteen days of antifungal

therapy (day +36), liposomal AMB was switched to oral therapy with voriconazole (200 mg twice daily). However, after two weeks (day +50), the patient developed a new lesion on the right knee and enlargement of two preexisting lesions, even though voriconazole levels in the blood were within the therapeutic range. Given the clinical deterioration, liposomal AMB was added to the therapy in combination with voriconazole. Biopsies of two further lesions were taken after 10 days (day +60) of combination antifungal therapy. Histological examination and culture of the biopsy specimens revealed no signs of fungal growth. Under combination therapy, the patient neither developed new skin lesions nor experienced enlargement of the pre-existing ones. However, on day fourteen (day +64) of the dual antifungal therapy, the boy began to show signs of acute kidney injury, and the use of liposomal AMB was discontinued, resulting in a rapid recovery of kidney function. The skin lesion transitioned from a nodule feature to a plaque one. During the hospitalization, the patient underwent lung High-Resolution Computed Tomography (HRCT) and abdominal Computed Tomography (CT), which ruled out signs of fungal infection in deep organs. On the other hand, the patient exhibited radiological signs of mucosal inflammation in the rhinosinus, which completely resolved 40 days later, as showed by Magnetic Resonance Imaging. Nasal swabs were collected and resulted negatives for filamentous fungi. Due to the complete resolution of inflammation, we decided not to perform an invasive procedure to obtain a sinus biopsy. Notably, the patient never exhibited elevated C-reactive protein (CRP) or β-D-glucan (BDG) levels, while other parameters during the all period of infection (Figure 2) are descriptive on table S1, nor was *Exserohilum* isolated from blood cultures. After discharge the lesions progressively improved.

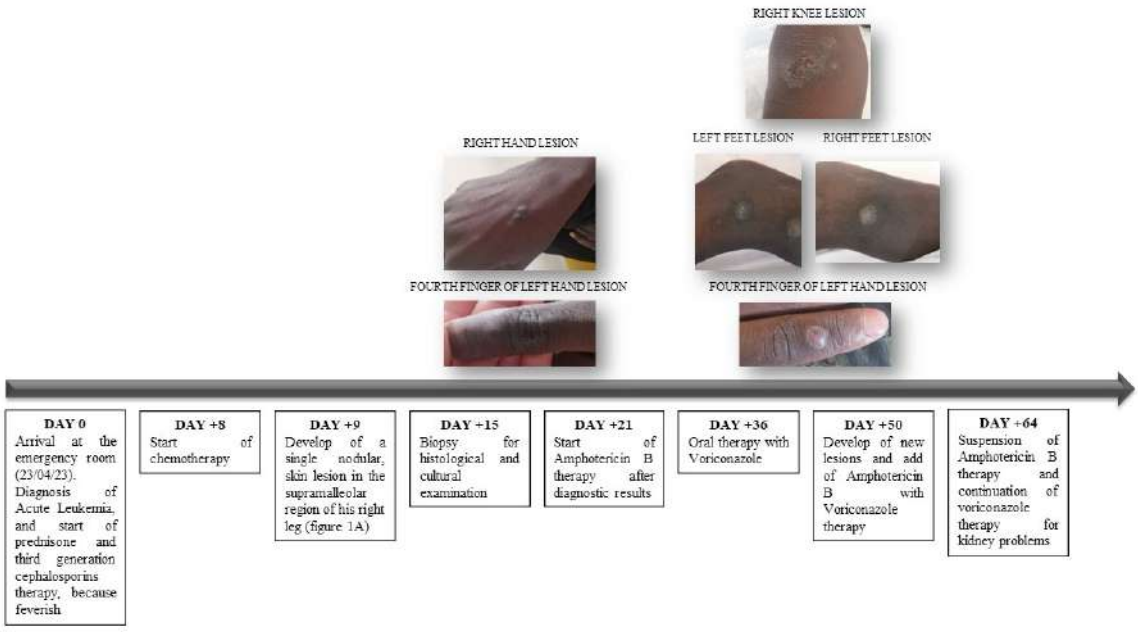


**Figure 2**: Illustrative flowchart about the entire infection period from day 0 to day 64 and images of minor skin lesion of *Exserohilum rostratum*.

| PARAMETERS | DAY 0 | DAY +21 | DAY +36 | DAY +50 | DAY +64 | Intervals of reference | IU |
|---|---|---|---|---|---|---|---|
| WHITE BLOOD CELLS (WBC) | 25,30* | 1,15* | 0,95* | 3,89* | 3,37* | 4,50-13,0 | 109/L |
| RED BLOOD CELLS (RBC) | 3,41* | 2,74* | 3,60* | 3,42* | 2,86* | 4,20-5,60 | 1012/L |
| HEMOGLOBIN (HB) | 10,5* | 8,3* | 10,9* | 10,1* | 8,4* | 12,1-16,6 | g/dl |
| HEMATOCRIT (HT) | 29,7* | 23,5* | 29,3* | 29,1* | 24,7* | 35,0-49,0 | % |
| PLATELETS | 200 | 28* | 66* | 407* | 57* | 140-400 | 109/L |
| LEUKOCYTE FORMULA | | | | | | | |
| NEUTROPHILS | 1,04* | 0,12* | 0,08* | 2,36 | 2,8 | 1,50-6,00 | 109/L |
| LYMPHOCYTES | 3,54 | 0,84* | 0,87* | 0,94* | 0,48* | 1,50-4,50 | 109/L |
| MONOCYTES | 0,25 | 0,02* | 0,00* | 0,54 | 0,09* | 0,10-1,30 | 109/L |
| EOSINOPHILS | 0,4 | 0 | 0 | 0,03 | 0 | 0,00-0,50 | 109/L |
| BASOPHILS | 0 | 0 | 0 | 0,02 | 0 | 0,00-0,20 | 109/L |
| PROTHROMBIN TIME (PT) | 1,17 | 1,11 | 1,21* | 1,35* | / | 0,8-1,20 | INR |
| S/P- GLUCOSE | 118* | 121* | 70 | 116* | 150* | 6-100 | mg/dl |
| S/P- CREATININE | 0,62* | 0,36* | 0,99 | 0,92 | 0,98 | 0,70-1,20 | mg/dl |
| S/P SODIUM | 133* | 134* | 138 | 135* | 133* | 136-145 | mMoli/L |
| S/P POTASSIUM | 4,2 | 4,2 | 3,5 | 3,6 | 3,1* | 3,5-5,1 | mMoli/L |
| ALANINE AMINOTRANSFERASE (ALT) | 9 | 56* | 22 | 39 | 51* | <41 | U/L |
| C-REACTIVE PROTEIN | 49,2* | 4,6 | 2,5 | 5,8* | 3,9 | <5,0 | mg/L |

Day 0= Therapy with third generation cephalosporins
Day +21= AMB therapy
Day +36= Voriconazole therapy
Day +50= Combined therapy with Voriconazole and AMB
Day +64= Voriconazole therapy
UI=International Unit

**Table S1:** Biologic parameters during the treatment including hematology, inflammatory markers and biochemistry.

## Discussion

The largest review of *Exserohilum spp*. human cases conducted in 2012 counted 48 cases from 1975. Out of them, only 8 involved pediatric hematological patients [6]. In the following decade, pediatric reports more than doubled in hematologic patients. We retrieved from the literature a total of 17 cases of *Exserohilum spp.* infections in children with hematological conditions from medical literature (Table 1).

| Reference | Year of report | Age/gender | Hematological conditions | Risk factor | Site of infection | Treatment | Outcome |
|---|---|---|---|---|---|---|---|
| Moneymaker [15] | 1986 | 3/M | ALL | Chemotherapy+ neutropenia | Skin | Surgical resection+ Flucytosine 2 weeks (wks) and AMB 4 ½ wks | Cured |
| Sharkey [17] | 1990 | 5/F | Aplastic anemia | Neutropenia | Sinus, soft tissue | AMB +itraconazole | Dead |
| Aquino [12] | 1995 | 7/F | Aplastic anemia | Neutropenia | Sinus+Lung | AMB + GM-CSF | Dead |
| Levy [13] | 2003 | 8/M | Relapsed ALL | Chemotherapy + neutropenia | Skin+sinus+lung | Surgery (skin lesions) + AMB +itraconazole | Dead (related to malignancy and Fusarium infection) |
| Adler [6] | 2006 | 8/F | ALL | Chemotherapy + neutropenia | Sinus | AMB then Voriconazole alone for months | Cured |
| Adler [6] | 2006 | 3/F | ALL | Chemotherapy+ neutropenia | Sinus and soft palate | Surgery+ AMB +itraconazole for months | Cured |
| Saint-jean [16] | 2007 | 3/M | ALL | Chemotherapy+neutropenia | Skin | Surgical +AMB 110 days | Cured |
| Juhas [18] | 2013 | 26 day/M | hemophagocytic lymphoistiocytosis | Steroids+Neutropenia | Skin | None | Dead |
| Dobinson [14] | 2018 | 7/F | ALL | Chemotherapy+neutropenia | Skin | Surgery+ AMB 2 wks then Voriconazole 6 mo | Cured |
| qaDobinson [14] | 2018 | 2/M | ALL | Chemotherapy+ neutropenia | Skin+lung+renal | Surgery+ AMB then Posaconazole 15 mo | Dead of bacterial infection |
| Dobinson [14] | 2018 | 2/M | ALL | Chemotherapy+neutropenia | Skin | Surgery+ AMB then voriconazole 2 mo | Cured |
| Dobinson [14] | 2018 | 8/F | ALL | Chemotherapy+ neutropenia | Rhinosinus | Surgery+ AMB 55 days | Cured |
| Dobinson [14] | 2018 | 6/F | ALL | Chemotherapy+neutropenia | Rhinosinus/ dissemination | Surgery+ AMB+CAS then voriconazole and terbinafine | Survived (at study publication) |
| Dobinson [14] | 2018 | 2/F | ALL | Chemotherapy+neutropenia | Rhinosinus | Surgery+ AMB 4 wks + Posaconazole 4 mo | Survived (at study publication) |
| Dobinson [14] | 2018 | 7/M | ALL | chemotherapy+neutropenia | Rhinosinus | Surgery+ AMB 3 wks+ voriconazole then posaconazole 6 wks | Cured |
| Pena [19] | 2019 | 1/F | ALL | chemotherapy+BMT | Skin | AMB B +voriconazole | Not known |
| Gracia-Darder [11] | 2020 | 3/F | ALL | No Chemotherapy initially | Skin | Surgery+ AMB 8 wks + voriconazole 22 wks | Cured |
| Present case | 2023 | 15/M | ALL | Chemotherapy+neutropenia | Skin, rhinosinus | Surgery AMB then voriconazole then AMB + vori then voricanozole alone | |

AMB= Amphotericin B, ALL: acute lymphoblastic leukemia.

**Table 1:** Summary of cases of fungal infections caused by *Exserohilum* species.

*Exserohilum* species are typically found in tropical climates. Pediatric cases have been reported from Australia, Israel, the United States, Canada, and Bolivia. The only case reported in Europe was in Spain. To the best of our knowledge, here we report the second case of infection ever described in Europe, involving a 15-year-old boy arrived from Senegal two months before the diagnosis was made [3,11].

Like adult patients, *Exserohilum* spp., infections in the pediatric immunocompromised population can cause a wide variety of clinical manifestations, being the skin and the sinus the most common sites of infections. Systemic dissemination has been reported in four children [12,13,14].

Skin infections were described as papules, nodules, plaques, or pustules, with initial presentation or as secondary lesions featuring ulcerations. Most of the lesions were located on the lower limbs. Some authors reported a preceding trauma as a risk factor for acquiring the infection [3,6]. In one case, cutaneous lesion developed in a skin area previously covered with cloth tape [15].

Regarding sinus localization the supposed route of transmission was by inhalation, but there are few data investigating this aspect further. Common risk factors in our case report were chemotherapy and neutropenia, accordingly with most clinical reports.

Diagnosis was consistently made through histology and microbiological culture on biopsy or swab specimens. No cases have reported *Exserohilum* growth in blood cultures [3,6]. Histologically, the appearance is commonly characterized by angioinvasive fungal aspects, however we were unable to report the histological images in the manuscript due to lack of availability by the reference pathological anatomy laboratory [6,14,16].

Next-Generation Sequencing (NGS) currently represents an important resource for achieving a precise identification of rare species or slow-growing fungal pathogens, reducing the time to result, and allowing for patient management. In some instance NGS represents the only resource to obtain fungal identification, interestingly in our case the identification was also obtained by mass spectrometry, nevertheless the rarity of this species [17-19].

The canonical therapeutic approach described in the literature typically involves surgical resection of skin and sinus lesions. Moreover, surgical excision is frequently combined with antifungal therapy with AMB, especially at the outset. In our case, after an initial empirical therapy with AMB, an azole has been adopted, either alone or in combination with AMB. In the literature, the most frequently utilized azoles were voriconazole, posaconazole, and itraconazole.

Katragkou et al. [3], reported an outbreak related to contaminated steroid injections, *E. rostratum* had low MICs for AMB, posaconazole, itraconazole, and voriconazole. Similar results were reported by a study published in the same year where AMB, itraconazole, posaconazole, and voriconazole had the lowest MIC [7].

In the literature, no fatal cases due to *Exserohilum* infections were reported. Antifungal therapies were prolonged for varying durations, ranging from weeks to months, typically until complete healing of skin lesions. No cases reported reactivation or relapse of *Exserohilum* infections after discontinuation of antifungal therapy.

In conclusion, here we describe a rare dematiaceous fungal infection in an immunocompromised pediatric patient with several risk factors for fungal infections, including leukemia and corticosteroid therapy, as well as traumatic factors such as skin injuries to the lower limbs that likely facilitated the entry of this rare fungus into the skin. Our patient likely acquired the infection in his country of origin, Senegal, considering the epidemiology of human infection of *E. rostratum* outside Europe. AMB therapy was crucial for resolving the lesions.

**Author Contributions:** Conceptualization, A.M., C.C., and L.C.; Software and data curation, S.Z. and G.D.; formal analysis and data curation A.M., G.G., M.S.M., M.B., A.M.DP.; resources and supervision M.C. and V.S.; writing-original draft preparation A.M., C.C. and L.C., and M.C.; writing-review and editing A.M., G.G., M.S.M., F.T., L.G., I.Z., M.B., C.B., M.C., and V.S.

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**Institutional Review Board Statement:** The following Case Report did not require approval from the Ethics Committee, but Informed Consent was requested and signed by the patient and the father, due to the patient’s age, after having received instructions on the possible risks and benefits and the rights were granted of privacy, confidentiality, and anonymity. The participant was free to refuse publication of their clinical data.

**Data Availability Statement**: Data supporting the study results can be provided followed by request sent to the corresponding author’s e-mail.

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**Conflicts of Interest**: The authors declare no conflict of interest.

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