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Case Report

First report of neonatal sepsis due to *Moesziomyces bullatus* in a preterm low-birth-weight infant

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Introduction: *Moesziomyces* spp. are connected with poaceous plants (Grass family), and *Moesziomyces bullatus* is commonly associated with smut in pearl millet. Currently, the scientific knowledge of this pathogen is limited to only a few taxonomic studies and there are no clinical reports that describe the isolation of *M. bullatus* from humans.

Case presentation: A female neonate born prematurely at 32 weeks of gestation was referred to the University Teaching Hospital in Jos, Nigeria, with a provisional diagnosis of preterm low birth weight at risk for sepsis. The birth weight of the newborn was 2000 g and her body temperature on admission was 34.3 °C. Blood cultures revealed the presence of a fungal isolate that was identified as *M. bullatus* by molecular methods. This fungus showed high MIC values for anidulafungin, caspofungin and micafungin, as well as fluconazole and 5-flucytosine, and exhibited varying degrees of susceptibility to itraconazole, amphotericin B, posaconazole and voriconazole. To the best of our knowledge, this is the first case of a human *M. bullatus* bloodstream infection.

Conclusion: Here, we report the first case of an unusual human infection caused by the fungal plant pathogen *M. bullatus* and highlight a high level of resistance to classical and modern antifungal drugs.

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Introduction

Moesziomyces spp. are connected with plants in the family of grasses (*Poaceae*), and *M. bullatus* is commonly associated with smut in pearl millet (Diagne-Leye *et al.*, 2010). *M. bullatus* is a basidiomycetous fungus in the order *Ustilaginales* and is phylogenetically closely related to the genus *Pseudozyma*, containing yeast-like fungi that are mostly epiphytic and are not pathogenic to plants (Buxdorf *et al.*, 2013). Several clinical cases including bloodstream and catheter-associated infections (Sugita *et al.*, 2003; Lin *et al.*, 2008; Prakash *et al.*, 2014) have been linked to different *Pseudozyma* spp. (Arendrup *et al.*, 2014), but there are no reports in the literature implicating *Moesziomyces* spp. in human disease.

Here we report, for the first time to the best of our knowledge, a case of *M. bullatus* infection in a preterm low-birth-weight infant, highlighting the diversity of smut fungi capable of causing opportunistic human infections.

Abbreviations: EBT, exchange blood transfusion; ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the sequence determined in this study is KF926673.

Case report

We describe a case of disseminated infection caused by *M. bullatus* in a female neonate born prematurely, the second

of a set of twins, at 32 weeks of gestation via an emergency cesarean section. The birth weight of the newborn was 2000 g and no anatomical abnormalities were observed on physical examination. The patient's body temperature on admission was 34.3 °C and fluctuated between 34.3 and 37.3 °C throughout her stay in the hospital. The white blood cell count at admission was 3100 mm^{-3} and afterwards oscillated between 3200 and 4500 mm^{-3}. The mother was a known hypertensive and had been treated with methyldopa and nifedipine. The newborn was delivered in a peripheral hospital and 3 h after birth she was referred to the University Teaching Hospital in Jos, Nigeria, with a provisional diagnosis of preterm low birth weight at risk for sepsis. During her 3 h trip from the regional clinic to the neonatal intensive care unit in Jos, the patient was exposed to non-sterile conditions. However, immediately after admission, blood cultures were collected aseptically for bacteriological analysis, and a combination of ampicillin plus cloxacillin and ceftazidime was commenced empirically. Significant clinical improvement (increase of plasma glucose level from 1.1 to 2 mmol l^{-1} and body temperature to 36.1 °C) was observed until day 3 of life, when the patient showed signs of marked jaundice. The neonate underwent exchange blood transfusion (EBT) on day 4 and responded well until day 9 when she developed jaundice again including bilateral pitting pedal oedema. She was then placed under phototherapy.

On day 10, the patient underwent another EBT, but her clinical condition clearly worsened and after a third EBT on day 19, her condition got worse.

Nasogastric tubes for feeding and intravenous catheters for delivery of antibiotics were changed at least twice weekly, whereas blood transfusions were carried out using separate catheters via the umbilical blood vessels.

In the initial blood culture, no bacterial pathogen growth was observed, although small (waxy and finely wrinkled) colonies were observed on the surface of blood and chocolate agar plates. Direct microscopic examination revealed the presence of septate hyphae that disarticulated to form arthroconidia compatible with identification as *Trichosporon* sp. Therefore, a new blood sample was collected and screened for bacterial and fungal pathogens. The antibiotic regimen was changed to cefotaxime, gentamicin and metronidazole. After 48 h of incubation, fungal colonies were again found on Sabouraud agar plates, especially on those incubated at 25 °C, and fluconazole was therefore empirically added to the ongoing pharmacotherapy. Nevertheless, the patient died on day 38 of life.

Phenotypic identification of this new isolate corroborated the initial assumption that the neonate was possibly infected by a *Trichosporon* sp.

Sequencing of the internal transcribed spacer 1 (ITS1)–5.8S–ITS2 region of the rRNA gene (White *et al.*, 1990) was performed to determine the identity of the fungal species. The sequence was used to search homologous sequences in GenBank and the results identified our isolate as *M. bullatus*. In fact, a high similarity was obtained with the sequence of GenBank accession number DQ831013 of the type strain *M. bullatus* CBS 425.34. Our clinical isolate and this strain differed by 0.15 % (1/666 nt). Phylogenetic analysis was also conducted to determine the relationship of *M. bullatus* to *Pseudozyma* spp., especially *Pseudozyma aphidis*, a potential human pathogen capable of causing life-threatening infections in humans (Lin *et al.*, 2008; Prakash *et al.*, 2014). Existing rRNA gene sequences were retrieved from GenBank and a phylogenetic tree was reconstructed. The resulting tree showed that our clinical strain clustered with the type strain of *M. bullatus* and formed a well-separated clade (probability 1/bootstrap value: 100 %) clearly distinguished from *Pseudozyma* spp. The distance of our strain from the type species of *P. aphidis* (CBS 517.83) and the other *P. aphidis* strains used was much larger. Comparing the two sequences, there were nucleotide differences including insertions/deletions. The variation between them was 7.3 % (50/686 nt in the alignment).

The fungal isolate has been deposited in the Centraalbureau voor Schimmelcultures, The Netherlands, as strain CBS 12827.

Further phenotypic characterization of *M. bullatus* CBS 12827 showed that our strain was able to grow over a wide range of temperatures (12–37 °C). The temperatures that gave maximum growth rate were between 24 and 33 °C with maximum growth at 27 °C. The fungal colonies grown on glucose/yeast extract/peptone agar appeared white to cream in surface colour and white/ivory on the reverse side after 7 days of incubation at 12 and 15 °C. Conversely, incubation at higher temperatures (18–37 °C) produced colonies that were white to pink with yellow to orange on the reverse side (Fig. 1a, b). The micromorphological characteristics of our isolate are shown in Fig. 1(c–f).

Evaluation of the *in vitro* activities of nine antifungal drugs was obtained using the Sensititre YeastOne Y010 microdilution method (Trek Diagnostic System) according to the manufacturer's instructions. The results revealed that *M. bullatus* CBS 12827 had high MIC values for anidulafungin (8 μg ml^{-1}), caspofungin (8 μg ml^{-1}) and micafungin (8 μg ml^{-1}) as well as for fluconazole (128 μg ml^{-1}) and 5-flucytosine (64 μg ml^{-1}). The MICs for itraconazole and amphotericin B were 0.12 and 1 μg ml^{-1}, respectively, but the values were at the limit of the susceptibility range. Low MIC values (0.03 μg ml^{-1}) only were obtained for posaconazole and voriconazole.

Discussion

Of the estimated 270 000 fungal species associated with plants, only a small subset can cross kingdoms and infect humans (Gauthier & Keller, 2013). Although these 'host

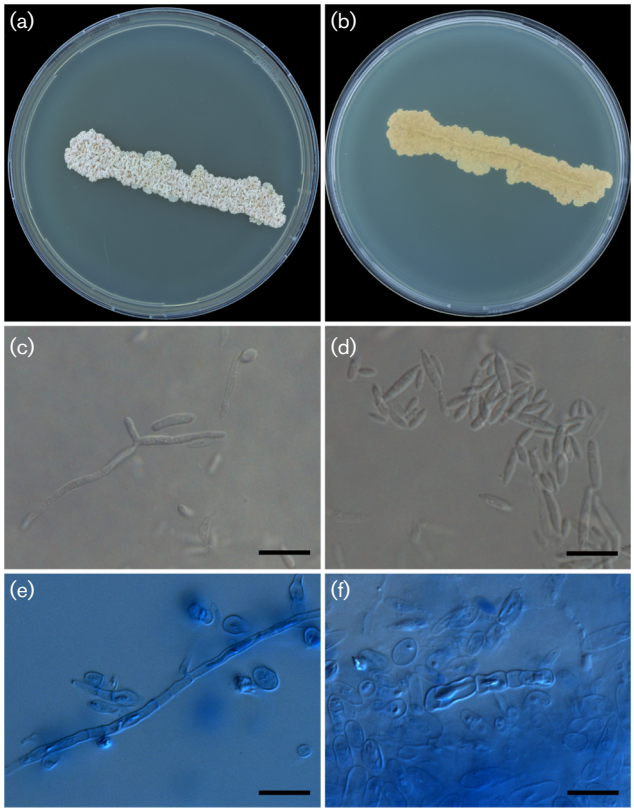

Fig. 1. Macroscopic and microscopic morphologies of *M. bullatus* CBS 12827. (a, b) Colonies were white to pink on top (a) and yellow to orange on the reverse (b) after subculture on glucose/yeast extract/peptone agar for 7 days at 25 °C. (c–f) Micromorphology showing growth of mycelium and blastoconidia (c), blastoconidia (d), formation of septa within the mycelium as the start of arthroconidia formation (e) and arthroconidia (f). Bars, 5 μm (c–e); 2.5 μm (f).

jumps' are very rare, several examples of fungal plant pathogens causing invasive human infections have been reported in the medical literature (Sharma *et al.*, 2014).

Retrospectively, in this study, it was difficult to determine how the infection was acquired. Potential sources included the maternal urogenital tract, external sources during transport to the hospital or acquisition during her hospital stay, such as via contaminated catheters. However, to the best of our knowledge, there are no other clinical reports that describe the isolation of *M. bullatus* from humans. A bibliography search, performed using PubMed with the term '*Moesziomyces bullatus*' retrieved no items and in general the scientific knowledge of this pathogen is limited to only a few taxonomic studies. Nevertheless, although several attempts have been made to clarify the taxonomy of different smut fungi, their relationship is not yet absolutely clear (Stoll *et al.*, 2005; Diagne-Leye *et al.*, 2010). In fact, combined analysis of the ITS and large subunit regions showed that *Moesziomyces* spp. appear to be paraphyletic, i.e. may not be within a well-separated genus (Stoll *et al.*, 2005). However, recent studies have indicated that the genus *Moesziomyces* falls in the same clade as the genus *Pseudozyma* and is closely related to *Ustilago* spp. (Diagne-Leye *et al.*, 2010). These latter two also contain human pathogens together with plant pathogens.

In this study, the repeated isolation of the fungus, in pure culture, from the blood, together with the lack of improvement of the patient's condition after empirical administration of fluconazole, led us to suspect that the fungus had multiplied within the host. In addition, antifungal susceptibility data revealed that our strain was highly resistant to fluconazole and therefore it was reasonable that the prescribed therapy was not efficacious.

Interestingly, it is noteworthy that infections caused by pathogenic smut fungi are associated with high levels of resistance to several antifungal agents (Arendrup *et al.*, 2014; Prakash *et al.*, 2014) and can result in serious and fatal outcomes.

Comparison of antifungal susceptibility profiles, obtained from *M. bullatus* and *Pseudozyma* spp. (Lin *et al.*, 2008; Arendrup *et al.*, 2014; Prakash *et al.*, 2014) showed remarkable similarity, especially with regard to resistance to the echinocandins, as well as fluconazole and 5-flucytosine. Therefore, according to Arendrup *et al.* (2014), voriconazole and amphotericin B seem to be a good option to treat *M. bullatus* infections given the low MIC values observed for these antifungals agents.

In conclusion, our case indicates that unusual fungi, such as *M. bullatus*, are also able to infect humans and therefore the total lack of studies on this species should stimulate future investigations in order to understand its basic biology including its genetics, virulence and pathogenicity.

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