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Fungemia due to the rare fungal pathogen *Kodamaea ohmeri* in a pediatric patient in Amazonia

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Abstract

Opportunistic yeast infections are increasingly recognized as contributors to child morbidity and mortality. *Kodamaea ohmeri* is an environmental yeast used in the food industry and a rare human pathogen. We report the case of a 3-year-old girl, in the Central Amazonian town of Manaus/AM, Brazil, diagnosed with fungemia due to *Kodamaea ohmeri*, who had a good outcome after antifungal therapy with amphotericin B deoxycholate and central venous catheter removal. At the end of the treatment and intervention the patient was discharged. The yeast was identified by biochemical tests and sequencing of internal transcribed spacer (ITS2).

Keywords: emerging pathogen, bloodstream infection, yeast, sepsis

Fungemia em paciente pediátrico, causada pelo raro patógeno humano *Kodamaea ohmeri* na Amazônia. Infecções oportunistas por leveduras são cada vez mais reconhecidas como contribuintes para a morbidade e mortalidade infantil. *Kodamaea ohmeri* é uma levedura ambiental usada na indústria alimentícia e um patógeno humano raro. Relatamos o caso de uma menina de 3 anos de idade de Manaus/AM, Brasil, com diagnóstico de fungemia por *Kodamaea ohmeri*, que apresentou boa evolução após terapia antifúngica com desoxicolato de anfotericina B e remoção do cateter venoso central obtendo alta hospitalar. A levedura foi identificada por testes bioquímicos e seqüenciamento do espaçador interno tanscrito (ITS2).

Palavras chave: patógeno emergente, infecção de corrente sanguínea, levedura, sepse.

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Introduction

Kodamaea ohmeri is a common environmental ascomycetous yeast that has been isolated from various substrates, including soil, plants, and insects (Bergman *et al.* 1998; Lee *et al.* 2007) and was transferred to the genus *Kodamaea* in 1995, having previously been placed in the genera *Pichia* and *Yamadazyma* (de Hoog *et al.* 2014). Currently, the genus *Kodamaea* comprises nine species: *K. ohmeri*, *K. anthophila*, *K. kakaduensis*, *K. laetipori*, *K. jinghongensis*, *K. neixiangensis*, *K. nitidulidarum*, *K. transpacifica*, and *K. meredithae* (Mycobank, 2018). Only *K. ohmeri* has been reported to cause infection in humans (Lee *et al.* 2007; Distasi *et al.* 2015). It is the teleomorphic (sexual) state of *Candida guilliermondii* var. *membranaefaciens*, which has long been considered a contaminant in hospital environments.

Since the confirmation of the first human case of fungemia due to *K. ohmeri* in the late 1990s, this yeast has been receiving growing attention and has been considered to be a major emerging pathogen due to its life-threatening potential to prolonged hospitalizations, preterm newborns, immunocompromised patients, and those using intravenous devices (Bergman *et al.* 1998; Distasi *et al.* 2015).

Most of the cases of *K. ohmeri* infection have been reported in Asia and, to date, very few are known from South America (Shang et al. 2010; Yamamoto et al. 2013; Chakrabarti *et al.* 2014). The only cases of *K. ohmeri* fungemia so far reported in this region consist of two patients from Northeastern Brazil and two from Northern Colombia (from Medellin and Bucaramanga) (Ostronoff et al. 2006; De Barros et al. 2009; Vivas et al. 2016; Alvarado-Socarras et al. 2016). In this study, we report a case of bloodstream infection caused by *Kodamaea ohmeri* in a 3-year-old patient admitted to a private children's hospital in Manaus, Amazonas state, Brazil. To the best of our knowledge, this is the first case report of *K. ohmeri* fungemia in the Amazon region.

Case report

A three-year-old girl was admitted to the pediatric emergency care at a private hospital in the city of Manaus, Brazil and diagnosed with mastoiditis. Three days prior to admission, the patient was having right retroauricular hyperemia, fever, ear pain, and left breast swelling and sought treatment at the same hospital. Despite antibiotic therapy with benzylpenicillin at the hospital and a 3-day oral antibiotic treatment at home, the patient showed no clinical improvement and evolved into serious infection of the right ear, with pain and pus-like discharge. On admission, the patient was treated with ceftriaxone (12 days) and subsequently with cefotaxime (5 days) and topical medications, and seven days post-admission underwent ear tube surgery with a positive outcome. The child remained hospitalized with fever and peripheral IV-related phlebitis. After 17 days of admission, a first blood culture was made and resulted negative. A central venous catheter was inserted to continue antimicrobial therapy with ceftazidime and clindamycin (7 days) and another 8-day antibiotic therapy with ceftazidime only. A second blood culture was made on the 18th day after admission, and *Kodamaea ohmeri* was identified in this second blood culture results on the 26th day after admission. The girl presented hyperemic lesions on her back spreading throughout the body, fever, and anasarca, and developed high fever peaks. Therefore, antifungal therapy was started on the following day with amphotericin B deoxycholate, and 6 days later all venous accesses were removed, and the antimicrobial therapy was interrupted. The patient remained hospitalized for 72 hours for observation and evaluation of leg pain and walking difficulty. The patient was discharged on the 35th day after admission and on the outpatient, follow-up visit one week later, the patient walked with no difficulty. Serological tests were performed for herpes and cytomegalovirus with negative results, but erythrocyte sedimentation rate (ESR) remained high.

Blood collection and fungal identification followed routine hospital procedures. Blood was drawn into Bactec

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pediatric bottles (BD, Paris, France) and cultured in a Bactec 9120 automated culture system (BD, Paris, France). The positive culture was grown on selective media (blood agar, MacConkey agar, Sabouraud's dextrose agar, and chocolate agar) and identified as *K. ohmeri* using the Vitek® system (bioMérieux, Marcy l'Étoile, France). Because *K. ohmeri* is not commonly found in blood culture, further tests were conducted in the Laboratory of Biodiversity in Health/Mycology at the Instituto Leônidas e Maria Deane (ILMD/FIOCRUZ), Manaus, Brazil. The yeast showed rough white-to-cream colonies with irregular edges when cultured on Sabouraud's dextrose agar (Figure 1) and colonies that changed color from pink to blue when cultured on CHROMagar *Candida* medium (Figure 2). Isolates were re-identified as *K. ohmeri* (99.9% probability) ID 6146376 using the API 20 C AUX (v 5.0) yeast identification system (bioMérieux SA, Marcy l'Étoile, France).

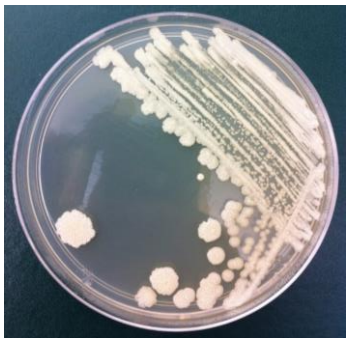

Figura 1. Macroscopic morphology of *Kodamaea ohmeri* grown at 30 °C on Sabouraud's dextrose agar (SAB) medium for 48 h, showing rough white-to-cream colonies with irregular edges.

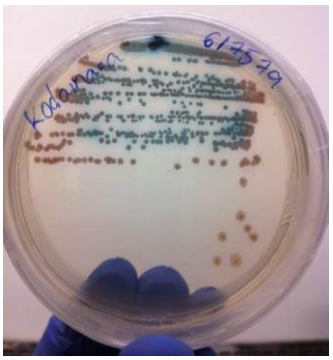

Figura 2. Macroscopic morphology of *Kodamaea ohmeri* grown at 30 °C on CHROMagar *Candida* medium for 48 h, showing the change in color from pink to blue.

Amplification and sequencing of the internal transcribed spacer (ITS2) were also performed. DNA extraction was done using the QIAamp Tissue and Blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Primers ITS3 (5'-GCATCGATGAAGAACGCAGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the ITS2 (Lee *et al.* 2007). All loci were sequenced in the forward and reverse directions using the same primers as those used for the PCR. Amplification of the ITS2 by PCR was conducted in a final volume of 25 µl, with each reaction containing ~40 ng of genomic DNA, 1x buffer solution, 2 mM $MgCl_2$, 0.4 mM deoxynucleoside triphosphates (dNTPs), 0.3 µM of each primer, 2 U of Taq DNA polymerase, and ultrapure water (q.s.). PCR was performed with the following conditions: initial denaturation at 96 °C for 1 min; 40 cycles of denaturation (96 °C for 30 s), annealing (60 °C for 30 s), and extension (72 °C for 45 s); and a final extension step at 72 °C for 7 min. The PCR products were purified using a 20% PEG-8000 solution and sequenced using an ABI 3130 Genetic Analyzer sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data were assembled using Geneious 7.1.9, and the finished sequence was used to search public databases (GenBank, EMBL and DDBJ) with BLAST (Megablast algorithm). Sixty-five hits presenting high scores revealed 100% similarity and coverage with *K. ohmeri*, with a score of 232.989 (Probability 2.36568E-60). In addition, a search was performed of databases maintained by the CBS-KNAW Fungal Biodiversity Centre (http://www.cbs.knaw.nl/) with the Pairwise sequence alignment tool using default conditions. Search results of 18 different databases found only *K. ohmeri*, with 100% similarity and overlap, and a score of 232. 989 (Probability 2.36568E-60). The nucleotide sequence obtained during this study was deposited in the GenBank nucleotide sequences database under accession number MN190177.1.

Discussion

To date, 57 cases of fungemia due to *K. ohmeri* in pediatric patients have been reported in the world, in addition to our case. All cases were linked to immunosuppression and use of invasive devices and the treatment was similar to fungemia due to other yeasts, as *Candida* spp. The antifungal resistance profile of *K. ohmeri* is variable, but resistance has been reported (Vivas *et al.* 2016).

Kodamaea ohmeri is a yeast that has been detected in water (Chakrabarti *et al.* 2014). In our case, it was reported that the child played in a water tank before becoming ill. It was not possible to test the water in question to confirm the presence of the yeast.

Epidemiological knowledge of yeast infections changes rapidly with various factors such as advances in fungal biology and taxonomy resulting from new molecular techniques, indiscriminate use of antimicrobial chemotherapy, more pervasive use of invasive medical procedures, and increased number of immunocompromised chronic patients (Ostronoff *et al.* 2006; Shang *et al.* 2010; Junqueira *et al.* 2012; Das *et al*. 2015).

As it has been documented elsewhere, teleomorph yeasts such as *Kodamaea* are less common in clinical specimens and require specific morphological testing and molecular analysis for proper identification (Hofmeyer and Slavin 2006). Unfortunately, most hospital laboratories are not adequately fitted for unusual fungi identification, thus resulting in treatment delay and worsening of patient conditions. Even when the microbial agent is properly identified, susceptibility testing methods are not standardized for non-*Candida* yeasts and resistance to fluconazole, amphotericin B, and caspofungin has been reported, what has led to further delaying the beginning of proper treatment (Passos *et al.* 2003; Hofmeyer and Slavin *et al.* 2006; De Barros *et al.* 2009; Chakrabarti *et al.* 2014).

Conclusion

This report highlights the need for special attention to be paid by practitioners and hospital staff in Amazonia to managing intravenous devices in order to reduce risk of opportunistic bloodstream infection. Hospitals in Amazonia should also incorporate more advanced molecular routines in their laboratories to improve specific diagnosis of fungal infections and better determine adequate antimicrobial therapy, thereby contributing to patient's prompt recovery.

Disclosure

This article is unpublished and is not being considered for any other publication. The authors and reviewers did not report any conflict of interest during their evaluation. Therefore, the *Scientia Amazonia* owns the copyrights, has the approval and the permission of the authors for disclosure, of this article, by electronic means.

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