

Kodamaea (Pichia) ohmeri fungemia in a pediatric patient admitted in a public hospital

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Kodamaea (Pichia) ohmeri is a yeast species that has not been reported to be a frequent cause of human infections. The current report describes a case of fungemia caused by *K. ohmeri* in a 3-year-old female patient hospitalized in the public hospital Maria Alice Fernandes, Natal, RN, Brazil. The patient had previously received antimicrobial therapy due to a peritoneal infection and nosocomial pneumonia, and had a central venous catheter implanted. *Kodamaea ohmeri* was isolated from blood and the tip of the catheter, 48 h after its implantation. The yeast was identified by standard microbiological methods and sequence analysis of the D1/D2 domains and the ITS 1+2 spacer regions of the ribosomal DNA. On CHROMagar Candida medium, the isolate showed a color change from pink to blue. The yeast was susceptible to amphotericin B, and liposomal AmB was used successfully to clear the infection.

Keywords *Kodamaea ohmeri*, fungemia, yeast, epidemiology, nosocomial infection, liposomal amphotericin B

Introduction

The incidence of fungal infections caused by a number of unusual fungi has been increasing among hospitalized patients during the last decades [1]. The use of broad spectrum antibiotics, catheters, transplantation, the occurrence of neutropenia, and depressed cellular immunity are factors usually associated with nosocomial fungemia [2]. *Kodamaea ohmeri* is an ascomycetous yeast, formerly classified in *Pichia*. The genus *Kodamaea* was separated from the genus *Pichia* because of a considerable genetic distance as measured by nucleotide sequence analyses of partial sequences of the 18S and 26S ribosomal RNA [3], and the monophyly of the genus was subsequently supported, e.g., in studies by Lachance *et al.* and Rosa *et al.* [4,5]. The genus

Kodamaea currently comprises five species: *K. anthophila*, *K. kakaduensis*, *K. laetipori*, *K. nitidularum* and *K. ohmeri* [6]. The latter is the only pathogenic species within this genus and causes rare infections in humans [7–21]. The species has also been isolated from various substrates, such as figs, dates, sambal ulak, gooseberry jelly, brines with various concentrations of salt and lactic acid, torani, salted cucumbers, rotten mushroom and seawater [22–24]. An accurate and rapid identification of pathogenic yeasts is essential for the optimal clinical management of infected patients [25]. Here we present a case report of *K. ohmeri* fungemia in a Brazilian child admitted in 2006 to a pediatric public hospital located in Natal, in the Northeast of Brazil.

Case report

A 3-year-old female child was admitted to the pediatric public hospital Maria Alice Fernandes (HMAF) in Natal, Brazil, on 24 May 2006. She was moved from another hospital with suspected pneumonia, paralytic ileum, and an infection by *Ascaris* spp. During admission to HMAF, she

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was treated with rocefin, metronidazole and mebendazole, and underwent colostomy. Clinical examination showed a pale patient in a severe state, internal globular abdomen, hematocrit 27%, leukocytes 5,100 per mm³, K 3.9 mg/dl, and Na 118 mg/dl. Two days after admission, she underwent a chest X-ray, which highlighted areas of atelectasis in both lungs. The patient was intubated due to discomfort to the lungs, received concentrated hemacia and was submitted for surgery of the abdomen. Gentamicin, vancomycin and imipenem were used as empirical antimicrobial therapy. On 19 June, the child was subjected to an exploratory laparotomy, characterized by an intestinal perforation caused by *Ascaris* spp. and an episode of purulent peritonitis. Eleven days after intubation, the child received a central venous catheter (CVC) to receive parenteral nutrition and drug administration. Forty-eight hours after implantation of the catheter, the patient developed an acute fever reaching a temperature of 38.2°C, which led to the removal of the catheter. The child received liposomal amphotericin B, because yeasts were isolated from blood samples and from the tip of the catheter. Fifty-six days after admission, the patient was discharged.

The samples from blood and the tip of catheter were sent to the microbiology laboratory of HMAF for identification and the presence of yeast colonies was revealed after subculturing on Sabouraud dextrose agar (Oxoid, Basingstoke, Hampshire, UK). The yeasts were identified using the ATB ID 32C (BioMérieux, Marcy l'Etoile, France) automated identification tool, and subsequently the identifications were confirmed by the laboratory of mycology of Federal University of Rio Grande do North, using morphological methods (i.e., germ tubes in bovine serum and chlamydospores on corn meal agar), cultivation on CHROMagar plates, and physiological tests. The yeast isolates were identified as *Kodamaea ohmeri*. No ascospores were seen after 15 days incubation on malt extract-2.5% glucose agar, thus confirming that the yeast isolates may be heterothallic, which is the most common form in which the species occurs. On CHROMagar medium, the colonies changed from pink to blue after 48 h and ID 32C identified the yeasts as *K. ohmeri* with 99% probability.

Genomic DNA for molecular studies was isolated as previously described [26]. Molecular identification of the isolate used sequence analysis of the D1/D2 domains and the ITS 1+2 spacer regions of the ribosomal DNA [27]. Basic Local Alignment Search Tool (BLASTn) [28] analysis of the ITS (FJ215865) and the D1/D2 (FJ215866) sequences in the NCBI database confirmed the identity of the yeast as *K. ohmeri*.

Susceptibility testing was conducted using the standard broth microdilution method as described in the CLSI M27-A2 document [29]. The concentrations of amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), itraconazole (Janssen Research Foundation, Beerse, Belgium),

voriconazole (Pfizer Central Research, Sandwich, United Kingdom) and posaconazole (Schering-Plough, Utrecht, The Netherlands) ranged from 0.016 to 16 mg/l, fluconazole (Pfizer Central Research) from 0.063 to 64 mg/l, anidulafungin (Pfizer Central Research) and caspofungin (Merck Sharp & Dohme BV, Haarlem, The Netherlands) from 0.008 to 8 mg/l and isavuconazole (Basilea Pharmaceutica, Basel, Switzerland) from 0.004 to 4 mg/l. *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 strains were included as quality controls. The MICs for the quality control strains were all within the reference ranges (data not shown). MIC endpoints of *K. ohmeri* were determined after 48 h incubation at 35°C both visually and spectrophotometrically at 450 nm (Anthos HT3, Salzburg, Austria) using the Mikrowin 2000 program. The MIC of amphotericin B was assessed as the well with the lowest concentration showing 100% inhibition of growth, while that of fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, caspofungin and anidulafungin were read as the well with a prominent decrease in turbidity (approximately $\geq 50\%$ growth inhibition) relative to the turbidity in the growth control well. MICs of the *K. ohmeri* isolate were 0.5 µg/ml for amphotericin B, 8 µg/ml for fluconazole, 0.125 µg/ml for itraconazole and voriconazole, 0.063 µg/ml for posaconazole, isavuconazole and anidulafungin and 2 µg/ml for caspofungin. No differences were noted between visual and spectrophotometric readings.

Discussion

The spectrum of infections caused by yeasts has undergone significant changes, and less common pathogenic yeasts have been reported as cause of nosocomial infections [2,30]. The use of broad spectrum antibiotics, CVCs, transplantation, and the occurrence of neutropenia and depressed cellular immunity are risk factors associated with nosocomial fungemia.

Here, we report a case of infection caused by *K. ohmeri* in a Brazilian three-year-old girl. Our case represents the second *K. ohmeri* related infection in Brazil. *Kodamaea ohmeri* is an ascomycetous yeast that usually occurs in the haploid state. The species occurs as an environmental yeast that has been isolated from various food-related habitats and seawater [22–24]. According to the medical literature, the first case of a *K. ohmeri*-related infection occurred in a 48-year-old, diabetic, insulin-dependent female patient with serious risk factors for immunosuppression [8]. So far, and including our case, 17 cases of *K. ohmeri* infection have been reported (Table 1). The other *K. ohmeri*-related infection in Brazil was related to a patient with chronic myelogenous leukemia [20].

In our case, liposomal amphotericin B cured the infection. This formulation has been used previously for the treatment of a *K. ohmeri* infection in a pediatric patient [17]. Interpretative

Table 1 Clinical features of Kodamaea ohmeri infections in the literature

Age, year/sex	Predisposing factors	Episode	Source	Treatment	Results	Location	References
48/F	Diabetes, kidney transplant	Fungemia	Blood, CVC	Removal catheter FCZ/Amb	Death	Iowa, USA	[8]
71/M	Pacemaker, endocarditis	Fungemia	Blood, CVC	Removal catheter, Amb	Death	Utrecht, NL	[10]
64/M	Peritoneal dialysis	Peritonitis	Peritoneal fluid	5-FC/Amb	Recovered	Hong-Kong, China	[11]
42/M	Hepatitis C, drug addict	Endocarditis	Blood	Vascular surgery AmB liposomal, 5-FC	Recovered	Almada, Portugal	[14]
76/M	Pacemaker, prosthetic valve	Endocarditis	Blood	AmB	Recovered	New York, USA	[12]
73/M	Lymphoma, chemotherapy	Fungiuria	Urine	FCZ	Recovered	Cádiz, Spain	[15]
84/M	Maxillary sinus carcinoma	Fungemia	Blood, CVC	Removal catheter Amb	Death	Ibaraki, Japan	[13]
59/M	Prolonged hospitalization, Peritonitis	Phlebitis	Blood	AmB	Recovered	Gwangju, Korea	[16]
14/M	Neutropenia, leukemia	Fungemia	Blood, PAC	Removal PAC	Recovered	Houston, USA	[9]
74/M	Infection of right buttock	Infection	Wound infection	Deep debridement	Death	Houston, USA	[9]
8 months/M	Encephalitis	Fungemia	Blood	FCZ	Death	Mersin, Turkey	[7]
10/M	Lymphoblastic leukemia	Fungemia	Blood, CVC	AmB	Recovered	Mersin, Turkey	[7]
Neonate/F	Premature	Fungemia	Blood	AmB liposomal	Recovered	Doha, Qatar	[17]
58/F	Chronic myelogenous leukemia	Fungemia	Blood, CVC	AmB	Recovered	Recife, Brazil	[20]
82/F	Diabetes, chronic renal failure	Fungemia	Blood	AmB	Death	Cádiz, Spain	[18]
–	Acute myelogenous leukemia	–	–	–	–	–	[21]
3/F	Intestinal drilling by <i>Ascaris</i> spp.	Fungemia	Blood, CVC	Removal catheter AmB liposomal	Recovered	Natal, Brazil	Present report

Amb, amphotericin B; FCZ, fluconazole; 5-FC, 5-fluorocytosine; PAC, Port-a cath; CVC, central venous catheter.

criteria for the susceptibility of *K. ohmeri* are not yet available, and limited data exist regarding the antifungal susceptibility pattern of this yeast. Nevertheless, the observed MIC values did not exceed those as accepted as susceptible for *Candida* according to the CLSI M27-A2 document. In our study, posaconazole, isavuconazole and anidulafungin showed the lowest MIC values, suggesting that these drugs may be active against strains of *K. ohmeri*. On the other hand, the observed lower susceptibility to fluconazole is in agreement with results reported by other authors [19]. The color change on CHROMagar Candida medium as observed in our strain has been reported in the past [18,19].

It can be concluded that infections by *K. ohmeri* occur in a broad range of patient categories, including neonates and children, and on different continents. Importantly, seven out of 17 cases were catheter related (see Table 1). Most likely, the number of described cases represents a low estimate of its actual incidence because it seems likely that the species may not be (properly) diagnosed in many routine hospital laboratories.

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