Cyberlindnera (Pichia) fabianii infection in a neutropenic child: importance of molecular identification

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Introduction: The number of fungal infections caused by uncommon fungi has increased in recent years. Cyberlindnera fabianii is a yeast species that is a rare cause of human infections. In previous cases, isolation of Cyberlindnera fabianii has been reported only from blood specimens and only infrequently. This report describes what we believe is the first case of isolation of Cyberlindnera fabianii from a urine specimen of an immunocompromised 5-year-old child who had a history of prolonged hospitalization and exposure to multiple antibacterial agents and who was neutropenic.

Case presentation: A 5-year-old male child presented in paediatric emergency in a febrile (103 °F), conscious but confused and irritable state. A number of risk factors were present in the child including an immunocompromised state, prolonged prior hospitalization, exposure to multiple antibiotics, indwelling catheters and neutropenia. A urine culture showed pure and significant growth of Candida sp., which was identified as Candida utilis (resistant to amphotericin B) by Vitek 2 Compact (bioMérieux). Subsequent 26S rRNA gene sequencing identified it as Cyberlindnera fabianii.

Conclusion: Molecular assays have a major role in confirming the identity of uncommon fungal isolates, as correct identification is important for epidemiological purposes. It is imperative that antifungal susceptibility should be performed along with identification of the Candida sp.

Key words: child infection; Cyberlindnera fabianii; neutropenia; urine.

Introduction
The incidence of yeast infections has increased tremendously in the past few decades (Arendrup, 2010). There has also been an increase in the number of yeast species being isolated. More than 17 different Candida spp. have been reported as aetiological agents of human infection and more species are being added. It has been reported that these uncommon, newly recognized species are associated with fungaemia, disseminated fungal infections, catheter-related infections and even onychomycosis (Abi-Said et al., 1997; Hazen, 1995). Candiduria or the presence of Candida in the urine, is a common occurrence in hospitalized patients and accounts for almost 10–15 % of nosocomial urinary tract infections (Jain et al., 2011). The most commonly isolated species from urine are Candida albicans and Candida glabrata. However, in patients who have not been exposed to a urinary catheter, Candida tropicalis and Candida glabrata are the most common. Other yeast species reported to cause urinary tract infections are Candida kefyr, Candida guilliermondii and Rhodotorula spp. (Hazen et al., 1999). Cyberlindnera (Pichia), a teleomorph stage of Candida spp., is ascomycetous yeast rarely involved in human infections. Here, we describe the isolation of Cyberlindnera (Pichia) fabianii from a urine culture of an immunocompromised 5-year-old male child, which was misidentified as Candida utilis by Vitek 2 Compact (bioMérieux).

Case report
A 5-year-old male child presented in paediatric emergency in a febrile (103 °F), conscious but confused and irritable state. His parents provided history that 3 months previously he had had a fever (intermittent) along with generalized clonic seizures for which he was admitted to another tertiary-care hospital. In that hospital, he was diagnosed as having tubercular meningitis with hydrocephalus. For management of the tubercular meningitis, antitubercular treatment was started and a ventriculoperitoneal shunt was put in to manage his hydrocephalus. During this period, the child also required intubation and was administered multiple antibiotics. He was also put on intravenous fluconazole as his urine culture showed significant growth of Candida sp. (the species was not identified).

The GenBank/EMBL/DDBJ accession number for the sequence of the Cyberlindnera fabianii isolate determined in this study is KP893553.
At the time of the current admission, his haemogram revealed 300 leukocytes mm\(^{-3}\) with 24 % neutrophils, indicating that the child was neutropenic. His haemoglobin was 9.4 g dl\(^{-1}\), serum bilirubin 0.8 mg dl\(^{-1}\), serum aspartate aminotransferase 52 IU l\(^{-1}\) and serum alanine aminotransferase 44 IU l\(^{-1}\). A coagulation study was normal. Microscopic examination of his urine showed the presence of abundant leukocytes and budding yeast cells. On culture, the urine showed growth of Candida spp. As it was isolated in pure form, in significant number (\(>10^5\) c.f.u. ml\(^{-1}\)) and was associated with the presence of pus cells and negative bacterial growth, the attending physician was informed and amphotericin B treatment was started immediately. Simultaneously, speciation and the antifungal susceptibility of the isolate were carried out using Vitek 2 Compact. The yeast was identified as Candida utilis (probability of 97 %) and was found to be sensitive to fluconazole, voriconazole, caspofungin and fluconazole but resistant to amphotericin B (MIC \(>4\) \(\mu g\) ml\(^{-1}\)). During this period, the condition of the child deteriorated and, before blood culture could be performed, the child died on day 2 of admission.

The isolate was sent to the Department of Microbiology, Post Graduate Institute of Medical Education & Research, Chandigarh, India, for confirmation of the species. It was identified as Cyberlindnera fabianii by 26 S rRNA gene sequencing using NL1 and NL4 primers (Wu et al., 2013) for the D1/D2 region. The sequence obtained showed 100 % homology with the reference sequence in GenBank (accession no. JQ342084.1).

**Discussion**

The VITEK-2 YST identification card showed that the isolated yeast was Candida utilis. Candida utilis is an uncommon Candida sp. that is known for its industrial application. It has rarely been described in humans and only a few cases of invasive human infections [fungaemia (Alsina et al., 1988; Bougnoux et al., 1993), fungal keratitis (Shih et al., 1999) and urinary tract infection (Hazen et al., 1999; Lukić-Grič et al., 2011)] caused by this organism have been reported to date. The rarity of Candida utilis as a human pathogen led us to perform molecular identification of the yeast. It was eventually identified as Cyberlindnera fabianii (also known as Lindnera fabianii or Pichia fabianii). As commercial yeast identification and diagnostic kits (e.g. API 20C AUX, ID32C and Vitek 2) used in routine practice have a limited ability to identify Cyberlindnera fabianii, this organism has also been misidentified as Pichia anomala or Candida utilis in other studies (Gabriel et al., 2012; Grenouillet et al., 2010; Hamal et al., 2008; Valenza et al., 2006). Yun et al. (2013) described a case of L. fabianii from the blood culture of a 47-year-old woman, which was identified initially as C. utilis by Vitek 2.

Cyberlindnera fabianii, a telemorph of Candida fabianii, is an uncommon human pathogen of low virulence. The present report describes the isolation of C. fabianii from the urine culture of an immunocompromised 5-year-old male child. To the best of our knowledge, in earlier cases, isolation of C. fabianii has only been reported from blood specimens and only infrequently. A review of the literature showed only seven case reports of fungaemia caused by P. fabianii worldwide: three from neonates (Bhally et al., 2006; Grenouillet et al., 2010; Wu et al., 2013) and four from adult patients (Gabriel et al., 2012; Hamal et al., 2008; Valenza et al., 2006; Yun et al., 2013).

Risk factors associated with Candida infection are diverse and are reported to be exposure to broad-spectrum antimicrobial agents, indwelling vascular catheters, prior surgery and cancer chemotherapy. In our patient, a number of these risk factors were present. The child was immunocompromised, had a history of prolonged hospitalization and exposure to multiple antibiotics, had been exposed to various indwelling catheters and was neutropenic. Neutropenia was also reported to be an important risk factor for L. fabianii fungaemia in one of the previous case reports (Yun et al., 2013).

Another unique finding in the present case report was that the isolated strain was resistant to amphotericin B. In previous cases, resistance to this antifungal agent has not been observed except for a case from China in which P. fabianii isolated from the blood sample of a premature infant had shown resistance to itraconazole and amphotericin B (Wu et al., 2013). A case report from Korea described clinical resistance of L. fabianii to amphotericin B, although the in vitro MIC value was 0.5 \(\mu g\) ml\(^{-1}\) (Yun et al., 2013). Thus, it is important to study antifungal susceptibility along with speciation of the Candida isolates.

The limitations of our study were as follows: (i) it could not be confirmed whether Cyberlindnera fabianii was a true pathogen and cause of mortality or an incidental finding, as a repeat sample was not available; (ii) the yeast isolate obtained from urine during the first admission was not determined to the species level, and thus it is not clear whether the infection was continuous or recurrent; and (iii) the source of infection could not be traced. As the child died within 48 h of admission, the infection was not acquired nosocomially from the present institution; however, it might have been acquired during the previous hospitalization.

In summary, we report the first case, to the best of our knowledge, of isolation of Cyberlindnera fabianii from a urine specimen of an immunocompromised 5-year-old child who had a history of prolonged hospitalization and exposure to multiple antibacterial agents and was neutropenic. As the isolate was found to be resistant to amphotericin B, it is imperative that antifungal susceptibility should be performed along with identification of the Candida sp. We also emphasize the role of molecular assays in confirming the identity of uncommon fungal isolates, as correct identification is important for epidemiological purposes.
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