

A case of *Candida metapsilosis* conjunctivitis in a neonate admitted in cardiac heart intensive care unit.

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Abstract

Nowadays apparently harmless *Candida* species especially rare ones, cause a rising number of infections. We report the first case of conjunctivitis due to *Candida metapsilosis* obtained from the eye discharge of 40-day-old girl with congenital heart disease admitted in the ICU. The isolate was resistant to fluconazole, anidulafungin and micafungin.

A case of *Candida metapsilosis* conjunctivitis in a neonate admitted in cardiac heart intensive care unit.

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Abstract

Nowadays apparently harmless and commensal *Candida* species specially, uncommon and rare ones, cause a rising number of infections although, very rare, but if they aren't treated, they can become serious and result in permanent consequences. *Candida* species can cause conjunctivitis after trauma and antibiotic therapy

to an inflamed eye. *Candida metapsilosis* is a recently described yeast, phenotypically indistinguishable from *Candida parapsilosis*, and molecular methods are essential for its detection. We report the first case of *Candida* conjunctivitis due to *Candida metapsilosis* obtained from the eye discharge of 40-day-old girl with congenital heart disease admitted in the cardiac intensive care unit (CICU). The yeast isolate was identified by traditional and molecular methods such as PCR-RFLP and sequencing of the entire *ITS* genomic region (ITS1, ITS2 and 5.8 rRNA). Antifungal susceptibility test performed according to the CLSI M27-A3, showed that the isolate was susceptible to amphotericin B, Itraconazole, voriconazole, clotrimazole, nystatin, terbinafine, 5-fluorocytosine and caspofungin whereas was resistant to fluconazole, anidulafungin and micafungin.

Keywords: *Candida metapsilosis*, pediatrics, uncommon *Candida*, Antifungal susceptibility test

Introduction

Due to an increasing number of predisposed patients and developments in medical interventions with increasing number of invasive medical procedures, some *Candida* species previously considered to be harmless commensals are emerging as causes of serious disease (1). Fungal eye infections are extremely rare, but they can be very harmful and may lead to blindness. Conjunctivitis is the most common eye infection and *Candida* species can cause conjunctivitis as a result of an eye injury or trauma; subsequently transmitted to the bloodstream (2, 3). *Candida parapsilosis sensu lato* is one of the common fungi causing conjunctivitis (1). *C. parapsilosis sensu lato* is ubiquitous yeast in nature and found in all environments. It is the most frequently colonizing species, isolated from the subungual space of human hands; therefore, can spread nosocomially through hand carriage. It has been frequently associated with infections in newborns (1).

Candida metapsilosis as a recently described *Candida* species is phenotypically and phylogenetically closely related to *Candida parapsilosis* species complex but is vary in geographic and anatomic prevalence, as well as in resistance characteristics (4). Using genotypic methods, the clonally related species of *Candida parapsilosis* complex was subsequently divided into three distinguish species: the more prevalent *Candida parapsilosis sensu stricto*, and two newly designate species *Candida orthopsilosis* and *Candida metapsilosis* (5, 6). It has been reported that 1–10% of the *C. parapsilosis* isolates identified through conventional biochemical tests are indeed *C. metapsilosis* or *C. orthopsilosis* (7).

We present a case of *C. metapsilosis* infection associated with conjunctivitis in a 40-day-old girl referred to Children’s Medical Center, undergoing heart surgery and admitted in Cardiac intensive care unit. To the best of our knowledge this was the first case of *C. metapsilosis* with no previously reported cases in Iran.

Case Report

A 40-day-old girl candidate for heart surgery was admitted to Children’s Medical Center in Tehran, Iran after diagnosis of congenital heart disease. She was treated with some related medicines and also sulfacetamide sodium ophthalmic solution in order to prevention and treatment of bacterial eye infections such as conjunctivitis. At the twelfth day of surgery and admitting in CICU, presented a discharge from left eye. Physical examination showed an eyelid swelling and erythematous conjunctiva with mucopurulent discharge from the left eye. Microbiological investigations were performed by culturing of purulent ocular discharge on blood agar, MacConkey agar and sabouraud dextrose agar (SDA), which yielded yeast in pure cultures and were reported as *Candida* sp. by performing the germ tube test (Reynolds-Braude Phenomenon). These isolates were subsequently cultured on CHROMagar *Candida* medium for purification and primary identification, and identified as *C. parapsilosis*. Also, the PCR-restriction fragment length polymorphism (PCR-RFLP) assay was described for rapid confirmation of identification.

DNA of yeast isolate was extracted using the boiling method (8). Briefly, three-four colonies of overnight culture were transferred to a 1.5 ml tube containing 50 μ l of sterile distilled water and placed in boiling water for 20 min, centrifuged for 10 min at 5000 r.p.m., and the supernatant was transferred to a new microtube and used as DNA template. and the ITS1-5.8S-ITS2 region was PCR-amplified (9). The PCR product was sequenced, and based on the sequence queries in the NCBI and ISHAM barcoding databases, the isolate

was identified as *Candida metapsilosis*. In vitro susceptibility of the isolate to amphotericin B, fluconazole, Itraconazole, voriconazole, clotrimazole, nystatin, terbinafine, 5-fluorocytosine, caspofungin, anidulafungin and micafungin was tested according to M27-A3 standard approach of clinical and laboratory standards institute (CLSI) (10), and the minimum inhibitory concentrations were 0.5, 2, 0.125, 0.015, 0.015, 1, 0.015, 0.063, 0.125, 8 and 8 $\mu\text{g}/\text{mL}$, respectively.

Written informed consent was obtained from the next of kin of the patient for the publication of any data included in this article.

Discussion

Candida parapsilosis sensu lato and its cryptic species has become the predominant causing candidemia in some pediatric settings, especially in newborns, it is responsible for 17-50% of bloodstream infections episodes (11) and the incidence of fungemia caused by *C. orthopsilosis* and *C. metapsilosis* have been increasingly reported in recent years (12, 13). *C. metapsilosis* is a rare entity in nosocomial *Candida* infections. Previous studies adjusted the frequency of infections attributed to *C. metapsilosis* from 0 to 35.5% of all *C. parapsilosis sensu lato* around the world (14). Studies suggested that *C. parapsilosis sensu stricto* and *C. orthopsilosis* are existed as human commensal, while *C. metapsilosis* is an environmental organism (1), accordingly the high rate of isolation of *C. metapsilosis* in a local area of China has been attributed to have similar exogenous origins (15). Nevertheless, we presented a case of *C. metapsilosis* associated conjunctivitis in a neonate, which to our knowledge there has been no reported to date, following an extensive literature search. Conjunctivitis is a term broadly used to describe an inflammation of the conjunctiva.

C. parapsilosis sensu lato that can be isolated from soil, plants, domestic animals, insects, seawater and marine environment; can be also isolated from, skin, gastrointestinal tract and mucosal surfaces including eye, vaginal and birth canal (1). That is one of the main species of the microflora of the subungual space (1). Therefore, there is two manners for a neonate to develop *C. parapsilosis sensu lato* conjunctivitis, one way is eye colonization from mother to neonate via the birth canal and post-antibiotic invasion after use the antibacterial drops in neonate settings, hence Colonization precedes infection; and another is the eye injury through the contact with the colonized hands and fingernail beds of the nursing staff (1). Accordingly, conjunctivitis caused by *Candida* species in newborn is a common occurrence (2, 16, 17). Anyway, transmission of the organism into the bloodstream and the development of candidemia is possible. A case of horizontal transmission of *C. parapsilosis* transmitted from the hands of two nurses to the neonate's conjunctiva and then to the bloodstream (2). Our finding suggests that *C. metapsilosis* can also be a human commensal, although, more researches are needed.

There are conflicting assessments in context of virulence and antifungal susceptibility pattern of *C. metapsilosis* (7, 11, 13, 18, 19). Nonetheless this species has been reported as the least virulent member of the *C. parapsilosis* complex, and the low frequency in clinical settings could be associated with this property and the disability of causing morphological changes and effects on the human cells and tissue culture models. Cell cultures exposed to *C. metapsilosis* releases the least hydrolytic enzymes and hemolytic factors, and represented the least biofilm production and pseudohyphae formation (14, 18). However, recent data indicated an increasingly important role of *C. metapsilosis* in human mycoses, especially in blood-stream infections, and its isolation from blood cultures deserves particular attention (15). It seems that a strain-dependent virulence mechanism might contribute to the invasiveness of this commonly non-virulent yeast (15)

It is remarked that accurate discrimination among cryptic species of *C. parapsilosis* seems unnecessary due to similar antifungal susceptibility profile to commonly used azoles and amphotericin B. However, concerning to echinocandins, identification to the species level is necessary because of different susceptibility patterns (15). Although It is reported that *C. orthopsilosis sensu stricto* is less susceptible to amphotericin B, echinocandins, and fluconazole than *C. metapsilosis* and *C. orthopsilosis*, Canton et al showed the high susceptibility of these two cryptic species to nine antifungal (13). Hence, accurate characterization and discrimination of these cryptic species are important in the two aspects of epidemiological surveys and antifungal susceptibility pattern.

Conclusion

Superficial infections by *C. metapsilosis* can be attributed to nosocomial transmission by hand carriage or vertical transmission. In this line of thinking, it could be a commensal organism and accurate identification needs to molecular methods such as ITS sequencing. Differentiation of this new species allowed to define the epidemiology and accurate treatment.

Author Contributions

SA performed all the experiments, data analysis, and interpretations. AC drafted the manuscript. AC and BN participated in collecting the clinical isolate and data collection. HE investigated the patient. HM was in charge for supervising the study and critical review of the manuscript. The final version of the manuscript was approved by all the authors.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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