Candida Pelliculosa Fungemia Cases in Pediatric Intensive Care Unit

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Abstract

Candida pelliculosa (teleomorph: *Wickerhamomyces anomalus*) is an opportunistic pathogen that rarely causes fungemia. Here we present two cases with *C. pelliculosa* fungemia in the pediatric intensive care unit. The fungemia developed as a result of horizontal transmission from a patient who was transferred from another hospital with the same condition. Strains isolated from blood cultures of the two patients were identified as *Candida pelliculosa* with API ID 32C (bioMerieux France) commercial kit. All strains were resistant to the same antibiotic and random amplified polymorphic DNA analysis type. E-test antifungal MIC levels were found in the order of 0.125 ug/mL, 24 ug/mL, 0.50 ug/mL, and 0.64 ug/mL for caspofungin, fluconazole, voriconazole, and amphotericin-B, respectively. Environmental samples for *C. pelliculosa* were negative. After measures to control the infection were implemented in the unit, the outbreak ended. In the case of hindering precautions, it should be taken into account that hospital-acquired infections can develop even from rarely encountered non-albicans *Candida* strains. *(J Pediatr Inf 2015; 9: 85-90)*

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Introduction

Historically, *Candida albicans* is the condition that is most frequently associated with fungal infections in humans. In recent years, with the advances in medical methods, the use of central venous catheter and antimicrobial agents has increased. As a result of this, the population of people with suppressed immune system has increased and the infection-causing yeast profile has changed. It has been reported in recent years that *non-albicans Candida* infections have emerged and the frequency of uncommon pathogenic yeasts (*Malassezia, Trichosporon, Hansenula*, *Rhodotorula* spp.) in nosocomial infections has increased (1). *Candida pelliculosa* (perfect form) and its telemorf form *Wickerhamomyces anomalus* (previously known as telemorf *Hansenula anomala* and *Pichia anomala*; gen bank anamorf *Candida beverwijkiae*) are the yeasts that generate ascospore (2). *Wickerhamomyces anomalus* has a widespread prevalence in the nature; it is prevalent in soil, plant material, various fruits, vegetables, tree exudations, leaves and other organic compounds (3, 4). *Candida pelliculosa* grows in mediums with high sugar very well, uses dextrose, maltose, sucrose and galactose and generates ascospore (3).

C. pelliculosa has been rarely reported to be the nosocomial fungemia agent in immunocompetent and immunosuppressed pediatric patients. Although many reported cases are composed of sporadic cases, fungemias in the pediatric intensive care units have been mostly reported as epidemics (5-7). Apart from the nosocomial fungemia, it has been reported to agent in intravenous-drug-use-driven candidemia in AIDS patients (8), meningitis agent in HVI patients (8), pneumonia (9), endocarditis (10), pancreatitis (11), ventriculitis (14) or urinary system (15) infections. Although primary reservoir of *C. pelliculosa* in the hospital environment

is not exactly known, the *C. pelliculosa* fungemia developing in pediatric patients has similar risk factors together with candidemia (5).

Colonization of *C. pelliculosa* in pediatric patients and the risk factors of these patients cause the development of fungemia (7, 12). Besides, transfer of the yeasts by the hands of healthcare workers causes catheter-related epidemics (6, 7, 14). In this report, the case of *C. pelliculosa* defined in two patients hospitalized in the Pediatric Intensive Care Unit (PICU) has been presented.

Case Reports

Case 1

A 2-month 10-day old male patient transferred to the PICU of our Faculty from an epicenter underwent a cardiovascular operation (coarctation of aorta) a month ago; afterwards *Candida spp.* growth was detected in the port culture while he was hospitalized in the intensive care unit due to pleural effusion development and fluconazole treatment was commenced. When he was admitted to our hospital, the patient who had high fever and infection symptoms was demanded peripheral blood culture and ampicillin-sulbactam+ amikacin treatment was initiated. Due to continuing high fever, the existing therapy was changed into meropenem treatment on the 4th day and upon the growth of non-albicans *Candida*, caspofungine was joined to the treatment. *Candida* continued to grow in the blood cultures taken from the patient on the 5th and 11th days. No yeast grown occurred in the 3 blood cultures afterwards; *Acinetobacter baumannii* and coagulase negative staphycoccus growth was detected. The patient died due to extended bleeding parameter and circulatory disorder and bradycardia.

Case 2

10 days after the first case, a 13-month-old female patient was hospitalized with the diagnosis of subarachnoid bleeding and cerebral edema. The patient who had a lesion on the left temporal region, infection symptoms and orientation loss was applied endotracheal intubation due to respiratory distress. KNS growth occurred in the peripheral blood culture taken on the third of her hospitalization. As a result of heightened fever on the eighth day, cefoperazone-sulbactam+amikacin+vancomycin treatment was started. Non-albicans *Candida* growth occurred in the blood culture (taken from the catheter) taken from the patient on the 12th day of her hospitalization. While no yeast growth afterwards occurred in the blood culture taken from the periphery, yeast growth was detected in the blood culture taken from the catheter. Patient's catheter was removed and repetition of candidemia was prevented. The patient followed up with the condition of cerebral hemorrhage was discharged at the end of treatment.

Mycology Studies

Identification

The cultures are inoculated to the Saboraud Dextroz agar (HIMEDIA, India) ve CHROMagar-Candida (RTA, Turkey) media from automatized blood culture system (BACTEC-9240/Becton-Dickenson, England) bottles that are monitored and the detected yeast cells on the Gram staining. Besides, another method of corn-flowered-tween 80 agar (Fluka, India) which we used routinely was left to be incubated on plates at 35°C through instillation planting (10µL substance is covered with suspension lamel left on the medium). The colonies that became visible after 48 hours in the planted medium became round, dry and dull in the SDA (Figure 1), and regular edged, purple in the middle and cream color on the outside in the CHROMagar-Candida (Figure 2). The hyphes started to be visible in the corn-flowered-tween 80 agara after 48 hour (Figure 3). The strains unable to be diagnosed definitively with these methods were defined as *C. pelliculosa* (profile no: 5244151111) via the API ID 32C (bioMerieux, France) commercial kit. For the verification of strains via genetic analysis, they were sent to Medical School Medical Microbiology Laboratory of Gazi University. The strains were defined as Wickerhamomyces *anomalus* (*C. pelliculosa*) as a result of ITS1 sequencing analysis.

In the screening samples taken from the PICU personnel and environmental sources by the infection control committee, no *C. pelliculosa* growth was found. As a result of the infection control measures taken in the intensive care unit, an epidemic was prevented.

Antifungal sensitivity test

Antifungal sensitivity of the *C. pelliculosa* strains was implemented via the E-test method in the Mueller-Hinton agar medium including 2% glucose and 0.5% methylene blue (21) (Figure 4). Therefore, the suspension prepared

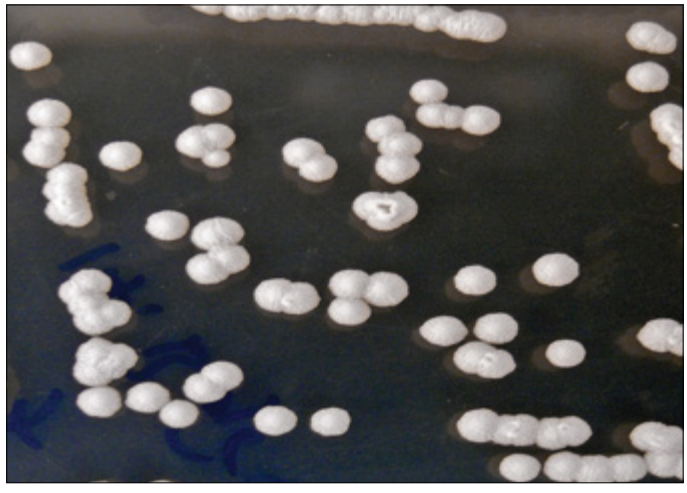

Figure 1. *C. pelliculosa colonies, SDAmedium*

according to 0.5 McFarland standard haze in every yeast strain inside every 0.85% sterile NaCl was inoculated onto agar surfaces, after the surface of the plated dried up, E-test stripes (AB bioMerieux, Sweden) kaspofungine (0.002-32 µg/mL), fluconazole (0.016-256 µg/mL), voriconazole (0.002-32 µg/mL) and amphotericin-B (0.002-32 µg/mL) were placed. The plates were incubated at 37°C for 24 hours, if there was insufficient growth, incubated for 48 hours and the results were evaluated.

Minimal inhibition concentration (MIC) sensitivity values for fluconazole (≤8 µg/mL sensitive, 16-32 µg/mL dose-sensitive, ≥64 µg/mL resistant) and for voriconazole (≤1 µg/mL sensitive, 2 µg/mL dose-sensitive, ≥4 µg/mL resistant) were implemented according to CLSI recommendations (22). MIC value for caspofungin (≤2 µg/mL sensitive) was evaluated based on the reference of a study (23). For amphotericin B (≤1 µg/mL sensitive, ≥2 µg/mL resistant) was accepted (24). According to the E-test results, MIC values of the *C. pelliculosa* strain for kaspofungine was found 0.125 µg/mL, for fluconazole 24 µg/mL, for voriconazole 0.50 µg/mL, and for amphotericin-B i0.64 µg/mL.

Figure 2. *C. pelliculosa colonies-CHROMagar Candida*

Randomly Amplified Polymorphic DNA (RAPD) Analysis

DNA isolation

The 3-day yeast colonies that grew in the SDA medium were subjected quick DNA extraction procedure (25). Yeast full of substance was dispersed in 1 ml sterile distilled water and kept at 80°C for 20 minutes allowing the tissues to be decomposed. Afterwards, the liquid left on the top was removed via centrifuge at 12,000 x g for 10 minutes. By adding 200 µl chloroform and 200 µl sterile distilled water onto the pellet, the mixture obtained was centrifuged again at 12,000 x g for 10 minutes. This time, the liquid left on the top was used as a pattern for PZR reaction.

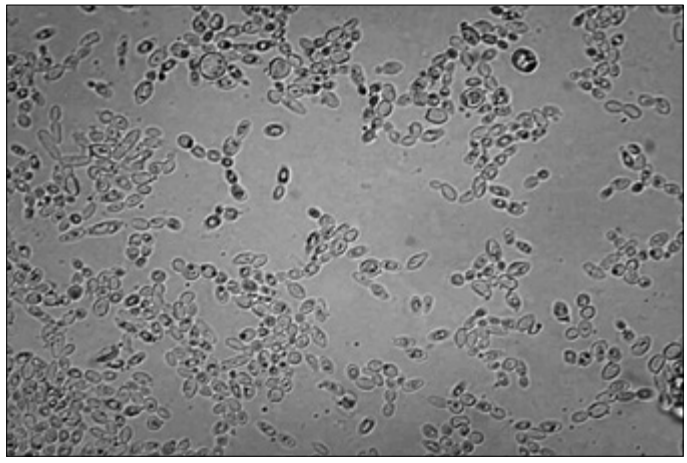

Figure 3. *C. pelliculosa,* instillation method, 48 hour later, corn-flowered-tween, 40X enlargement

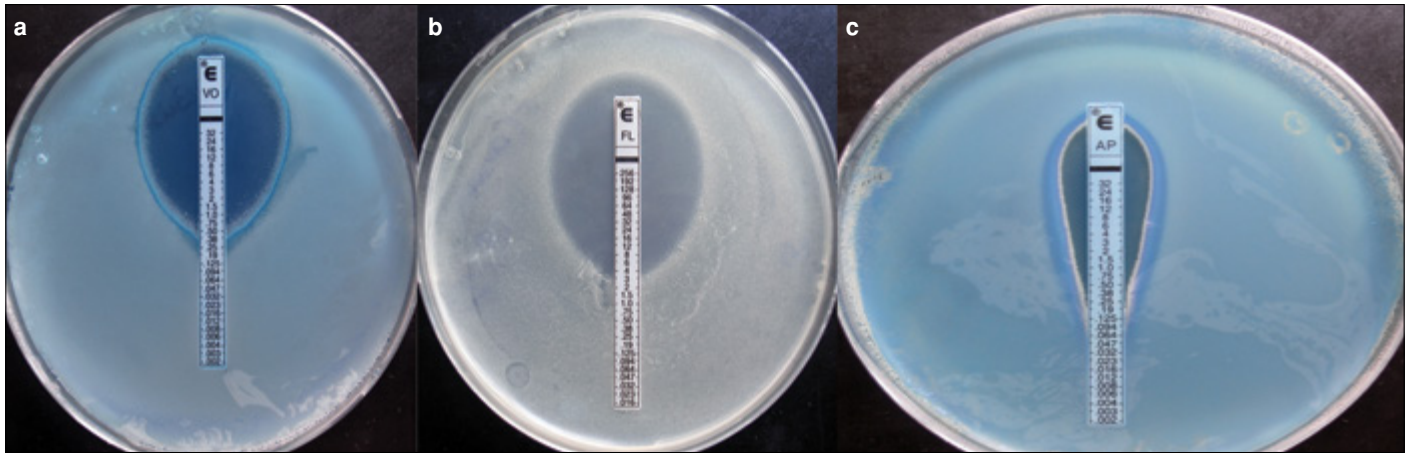

Figure 4. a-c. 5% methylene blue MHA, E-test (AP: amphotericin-B; VO: voriconazole; FL: fluconazole)

RAPD-PZR

In order to determine the epidemiologic relationship of the 6 *C. pelliculosa* strain isolated in both patients, RAPD-PZR analysis was implemented via M13 primary. As a result of the analysis, it was found that all the strains were epidemiologically related (Figure 5).

RAPD analysis: For each *C. pelluculosa* strain, 50 µl RAPD-PZR mixture was prepared; 2 µl genomic DNA, 100 pmol M13 primary (5'-CAG GGT CCC GGT TCT-3'), 2,5 U Taq DNA polymerase, 10 mmol dNTP mixture, 5 µl 10X buffer and 2.5 mM $MgCl_2$. The amplification procedure implemented for the reaction mixture is as follows; 2 cycles, 5 mins. at 94°C, 5 mins. at 40°C, 5 mins. At 72°C; and 40 cycles, 1 min. at 94°C, 1 min. at 40°C and 2 mins. at 72°C. The products amplified in the thermal cycler device (Eppendorf) were subjected to agarose jelled electrophoreses inclusive of 1.5% ethidium bromur and was viewed in the UV-illuminated device (26).

Sequencing Analysis of ITS Gen Region

In our study, amplification of the ITS region was carried out by using the ITS 1 (forward): 5-TCC GTA GGT GAA CCT GCG G-3 and ITS 4 (reverse): 5-TCC TCC GGT TAT TGA TAT GC-3 universal primaries. For the purpose of purifying amplified PZR products, ExoSAP-IT purification kit was used. Afterwards, the purified products, DNA sequencing analysis reaction was prepared in accordance with the usage recommendation of the BigDye BigDye® Terminator v3.1 Cycle Sequencing DNA sequencing analysis kit. DNA sequencing analysis of the amplified products was realized by the ABI Prism ™ 310 Genetic Analyzer DNA Sequencing Analysis Device (Applied Biosystems, ABD). Data was analyzed by the Sequencing Analysis 5.3.1 program. The sequencing analysis data obtained through capillary electrophorus was evaluated by using the National Center for Biotechnology Information (NCBI, Bethesda, Md., ABD) BLAST system. As a result of the sequencing analysis of the derivatives, they were defined as 100 % *Wickerhamomyces anomalus.*

Discussion

Candida pelliculosa is yeast often isolated in the telemorf *Wickerhamomyces anomalus* dough yeasts and laboratory yeast fermentations (27). The very first infection report related to *C. pelliculosa* is the publication by Wang and Schwarz in 1958 in which it was revealed that it was isolated in the lung of infants (19). The first neonatal intensive care unit epidemic report was published in 1986. It was found that in 13-month period, 52 newborn infants were colonized with *C. pelliculosa* and 8 infants were infected. It was reported that fungemia developed in five

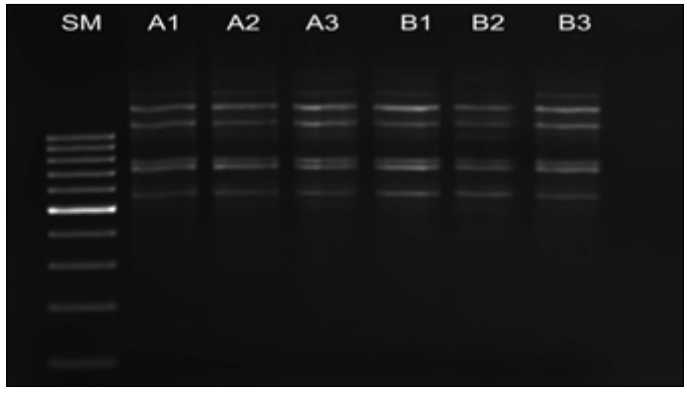

Figure 5. Jell electrophorese image of *Candida pelliculosa* strains after RAPD-PZR. A1, A2, A3: Strains isolated from patient 1; B1, B2, B3: Strains isolated from patient 2

infants, fungemia and ventriculitis in two infants and ventriculitis in only one infant. The infected infants were treated with flucytosine and amphotericin-B combination; however, elimination of the yeasts was enabled via oral nystatin prophylaxis and iodophor application in the intravenous access site. Researchers stated that no reservoir except the infants was detected (12). According to Kalkancı et al. (6) report in 2010 including the literature review on *C. pelliculosa*, 31 *C. pelliculosa* cases and epidemic was reported. In the following periods, two case reports (meningitis in the HIV patients and fungemia in sickle-cell anemia) and 2 neonatal intensive care unit epidemic reports were included in the literature (8, 20-22). According to the data, 11 epidemic and 24 case reports were reported and 9 were detected in the pediatric unit and 2 in the adult patient unit. While *C. pelliculosa* fungemia cause more epidemics in the pediatric units, it is noticeable that it occurs in the form of sporadic cases in adults (6).

The potential risk factors reported in the *C. pelliculasa* infections in pediatric patients are low pregnancy age, low birth weight, extended hospital stay, premature birth-related problems, parenteral nutrition, antibiotic treatment and invasive procedures (5, 7, 12). However, in the suspicion of exogen infections, central venous catheter and the length of stay in the intensive care unit were reported to be the most important risk factors; besides, it was found that *C. pelliculosa* fungemia risk factors and the candidemia risk factors caused by other *Candida* species were the same (5). In the *C. pelliculosa* epidemic report including 24 cases in an oncology hospital in Brazil, it was reported that average age of the patients was 11 years, the most underlying disease were leukemia (62.5%) and lymphoma (25%) and all the patients had central venous catheter or peripheral venous catheter (14).

In neonatal ICU epidemics, Murphy et al. (12) found that in 13-month period, skin and mucosa (skin, rectum, oropharynx) of 52 neonatals (10% of the patients admitted to ICU) were colonized with *C. pelliculosa* and infec-

tion developed in 8 infants. In another study, *C. pelliculosa* colonization (abdominal region, mouth, rectum) was found in 14 (28%) of the 50 premature neonatals and it was reported that fungemia developed in 20% of them (7).

The basis of the spread of yeasts in nosocomial *C. pelliculosa* epidemics is stated that it may be as a result of cross contamination through the hands of healthcare personnel from the first contaminated or infected patient at the hospital. However, this is difficult to prove unless patients are scanned at their admission (6, 7). In their study, Chakrabarti et al. (9) revealed through the hand culture positivity that the spread of *C. pelliculosa* strains from the pediatric emergency to the neonazal ICU could be transmitted through the hands of the healthcare personnel.

In this study, the first case was a 2-month 10-days-old infant who was transferred to PICU of our hospital from another health center. The patient came from an epicenter with *Candida spp*. growth in the port culture; due to high fever, blood culture was taken and through conventional methods and within the expected period of time, unidentified non-albicans *Candida* (*tropicalis, parapsilosis, glabrata, kefyr, non-krusei*) growth occurred. The yeast defined as *C.pelliculosa* at the end of 48 hours incubation via the API ID 32 C commercial kit, was detected in two blood cultures as well. KNS growth was found in the blood culture of the 13-month-old female patient taken on the 3rd day hospitalized in the same unit 10 days later. It was realized that yeast colonies whose morphological similarities were similar to C*. pelliculosa* grew in the blood culture taken from the catheter on the 12th day due to high fever. Accordingly, a passage was repeated in the blood culture bottle taken from the patient on the 3rd day and it was observed *C. pelliculosa* together with CNS grew.

The difficulties experienced in defining the rarely isolated infection agent/colonized yeast fungus through routinely used conventional methods may delay the treatment-initiating results. Therefore, it is an important approach to make the correct definition of the specie in yeasts and *Candida* species in terms of initiating the correct treatment.

C. pelliculosa could not be isolated in the environmental screening sampled carried out by the infection control committee with the second case. Research results led us to think that the first strain was carried from an epicenter by the first case and transmitted to the second case through cross-contamination via the healthcare personnel. It was found as a result of molecular epidemiologic analysis done by RAPD-PZR that the six strains isolated from the two cases were clonally related to each other. After increasing the infection control measures, no more *C. pelliculosa* fungemia was detected in the PICU.

The risk factors identified in the two cases in our hospital such as weak immune system, respiratory insufficiency-related intubation, congenital cardiovascular problem in a case and ensuing operation, cerebral edema in the other case, antibiotic treatment, central venous catheter and long stay in the intensive care unit, are similar to the risk factors in the *C. pelliculosa* reports.

It was reported that in most of the *C. pelliculosa* cases, removing the catheter and amphotericin-B treatment created successful results in infections (6, 23). Kalkancı et al. (6) reported that *C. pelliculosa* fungemia was successfully treated by amphotericin-B, fluconazole and removal of the catheter. It was found in the in vitro test in the same study that *C. pelliculosa* strains were weakly sensitive towards fluconazole, but were sensitive to amphotericin-B, itraconazole, voriconazole, myconazole, flucytosine and mikafungine. Barchiesi et al. (4) reported that 46 *C. pelliculosa* strains isolated in 37 patients had low sensitivity to azoles; 3 strains isolated in a patient were resistant to flucytosine, but all the strains were sensitive amphotericin-B. It was emphasized in the same study that the most appropriate antifungal for *C. pelliculosa* cases could be amphotericin-B. It was revealed that the strains isolated from two cases in this study were sensitive to amphotericin-B, caspofungin, voriconazole; and weakly sensitive to fluconazole/dose-sensitive.

Conclusion

In conclusion, although *C. pelliculosa*, is an isolated yeast as a rare an infection agent, it has been shown that yeasts can be transmitted in the infected hosts via the transfer of the patients between the health centers and as a result of cross-contamination, infection-related epidemics can occur in new hosts. In order to be able to prevent the transmission ways of nosocomial yeast infections in the pediatric intensive care units, the present study has emphasized once again that infection control measures should be scrupulously implemented.

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