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Case Report

Non-*albicans* Yeast Scalp Infection in a 6-Year-Old Boy: A Case Report of *Candida parapsilosis* and Antifungal Susceptibility Testing

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ABSTRACT

Background and aims. *Candida albicans is implicated in most human superficial and mucosal infections, although, other species such as C. glabrata, C. tropicalis, C. parapsilosis, C. kefyr, C. krusei, and C. gulliermondii may also be involved. The most common affected sites include the face, skin, scalp, and hands. The study aimed to identify the causative agent and determine the antifungal susceptibility testing (AFST) profile of the isolate responsible for scalp infection for proper assessment of management response.* ***Case report****. We describe a case of a 6-year-old boy, who has a scalp infection caused by Candida parapsilosis, with symptoms of itching, dryness, round patches and alopecia. Empirical treatment using 1% hydrocortisone and later reviewed to betamethasone 0.1% lotion were not successful. The patient was successfully treated with 2% ketoconazole topical ointment for 8 weeks after diagnosis.* ***Results****. The isolate showed variable minimum inhibitory concentration results among the antifungal drugs (azoles and echinocandins) tested. The isolate demonstrates high MIC values ≥ 3 and ≥ 4 µg/ml to newer triazoles posaconazole and itraconazole respectively, despite posaconazole being a second-generation triazole. Moreover, in the echinocandins class, micafungin showed a low MIC result (≤ 0.19 µg/ml), then followed by anidulafungin (≤ 2 µg/ml) and caspofungin showed resistance (≥ 32 µg/ml). Amphotericin B (polyene) showed a low MIC value (≤ 0.64 µg/ml) and flucytosine indicated a MIC value of ≤ 0.5 µg/ml. Ketoconazole and voriconazole indicated low MIC (≤ 0.125 µg/ml and ≤ 0.64 µg/ml respectively) and therefore, guided the therapy because of the ketoconazole availability and its cost-effectiveness.* ***Conclusion.*** *These findings highlight the importance of recognizing the increasing observance of C. parapsilosis strains in scalp infection. The patient responded to treatment with hair started developing after the sixth week of therapy and the patient fully recovered at the end of the treatment.*

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INTRODUCTION

A limited number of *Candida* species such as *C. albicans*, *C. glabrata*, *C. dubliniensis*, and *C. parapsilosis* are associated with the colonization of a human host. When the condition is favourable, these species can cause infection from

superficial to fatal invasive candidiasis [1]. Among the *Candida* species, *C. albicans* and *C. glabrata* ranked first and second, respectively in the frequency of isolation. Together, they are responsible for approximately 65–75% of all systemic candidiasis, followed by *C. parapsilosis* and *C. tropicalis* [2]. Although *C. albicans* is the most prevalent species in the genus *Candida*, non-*albicans* species have also stolen the limelight. They are frequently isolated at an increasing rate. In recent years, *C. parapsilosis* has received dramatic attention. It has been reported to be the third most commonly isolated *Candida* species from blood cultures in the USA [3]. *Candida parapsilosis* is a typical human commensal of healthy individuals, and its pathogenicity is checked by intact integument [4]. *Candida parapsilosis* is notorious for its capacity to grow in total parenteral nutrition and form biofilm on catheters and other implanted devices, for nosocomial spread by hand carriage persistence in the hospital environment [5]. Accurate identification of the causative species is essential since different *Candida* species have been documented to have antifungal drug resistance. The worldwide incidence of fluconazole resistance in *C. parapsilosis* invasive candidiasis ranges between 2 and 5%. Azoles are preferred antifungal drugs for treating infections caused by species in the *Candida* genus, for their low toxicity and oral administration availability [6]. Here, we report a case of *C. parapsilosis* infection from an immunocompetent individual, possibly acquired through laboratory handling. The study aimed to identify the causative agent and determine the antifungal susceptibility testing (AFST) profile of the isolate responsible for scalp infection for proper assessment of management response.

CASE REPORT

A 6-year-old boy was presented to a dermatologist for a scalp infection. His parents described the symptoms of itching, loss of hair and round patches on the scalp skin that appeared 1-month ago **(Figure 1)**. On day 1: He was empirically treated with a prescription of 1% hydrocortisone twice daily for 3 weeks pending the outcome of the laboratory results. The patient failed to experience any improvement after the treatment. After 3 weeks, the treatment was reviewed to a more potent betamethasone 0.1% lotion twice daily for 2 weeks with no success. The dermatologist requested laboratory investigations to unravel the identity of the causative agent and its drug susceptibility profile. The treatment was further reviewed to 2% Ketoconazole (Nizoral ointment) based on AFST results for an extended period of 8 weeks.

After the failure of empirical treatment; a skin scraping sample of the affected scalp was taken and cultured on sabouraud dextrose agar (SDA) (Merck, Germany). The causative agent was identified via the process (culture, gram stain, PCR using ITS1/ITS4 primers) and confirmed by DNA sequencing. Antifungal susceptibility testing was performed on the recognized *C. parapsilosis* isolate using the E–test method (Liofilchem, Italy) on Mueller-Hinton agar supplemented with 2% glucose and 0.5 μg/ml methylene blue. Nine (9) antifungal agents were tested as indicated in **Table 1**. For comparison, three antifungal agents (fluconazole, amphotericin B and caspofungin) representing the major antifungal drug classes (azoles, polyene and echinocandin respectively) were tested using broth microdilution (BMD) reference method (CLSI, 2008a) [7] for AFST evaluation on the isolate. Accordingly, a 100 μl of RPMI 1640 medium containing 0.165 M morpholinepropane sulphonic acid (MOPS) buffer, without bicarbonate, 2-fold dilution of each drug and 100 μl of 0.5×10^3 to 2.5×10^3 cells per ml of *C. parapsilosis* inoculum were added per well in 96 well plates.

A comparative evaluation of the E–test method and gold standard BMD assay on *C. parapsilosis* against the antifungal agents was conducted. The MIC reading for E – the test method was determined based on the position where the inhibition eclipse intersects the strip. The MIC was read and evaluated on the strip gradient scale as indicated in Fig. 3 by the same two investigators with a third investigator designated to resolve results discrepancies. The MIC endpoints were determined based on the CLSI (M27-A3) guidelines for the broth microdilution method, after 24 h incubation at 35°C. The MIC results for both methods were compared with MIC breakpoints of CLSI (M27-A3) for *in vitro* susceptibility testing of *C. parapsilosis* (Table 4).

RESULTS

The patient responded well to treatment using 2% Ketoconazole (Nizoral ointment) with hair started developing after 6th week of therapy initiation and fully recovered at the end of the treatment (8th week). Pasty colony appearance on the culture plate together with the microscopic appearance of oval-shaped yeast in 20% KOH and observed Gram-positive (purple) budding yeast give a presumptive cause as yeast (**Figure 2**). The conventional PCR band size (495 bp) (**Figure 3**) and DNA sequencing result confirmed the identity of *Candida parapsilosis* through NCBI blast and is 99% similar to KX652405 deposited sequence. The isolate indicates variable MIC values, within each class (azoles and echinocandins) of the antifungals tested. In azoles, only ketoconazole and voriconazole indicated low MIC ($\leq$ 0.125 µg/ml and $\leq$ 0.64 µg/ml respectively), while posaconazole and itraconazole showed high MIC values $\geq$ 3 and $\geq$ 4 µg/ml respectively, despite posaconazole being second generation triazole. Moreover, in the echinocandins class, micafungin showed a low MIC result ($\leq$ 0.19 µg/ml), then followed by anidulafungin ($\leq$ 2 µg/ml) and caspofungin showed resistance

(≥ 32 µg/ml). Amphotericin B (polyene) showed a low MIC value (≤ 0.64 µg/ml) and flucytosine indicated a MIC value of ≤ 0.5 µg/ml.

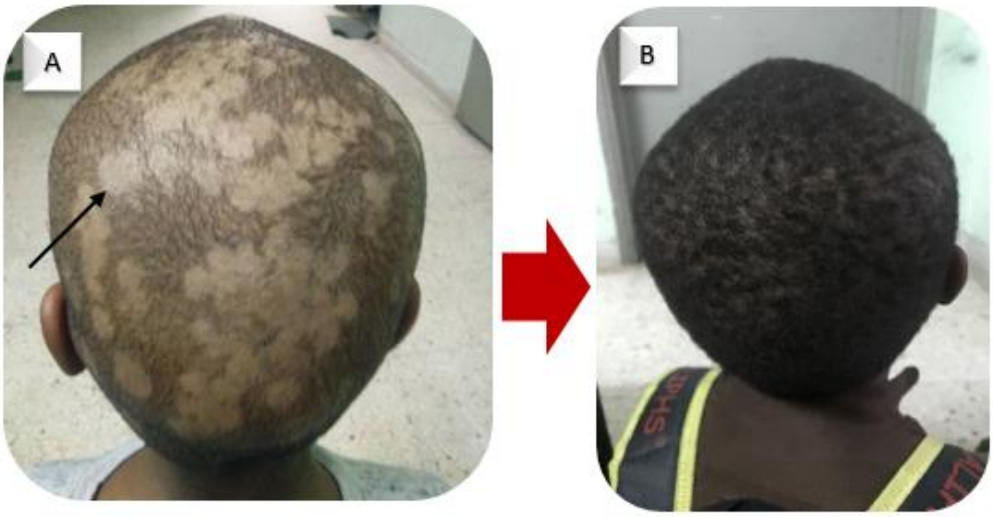

Fig. 1: Legend: *Candida parapsilosis scalp infection* ***(A):*** *(Arrow) shows round patch area of alopecia before treatment;* ***(B):*** *No patches or scales observed after treatment.*

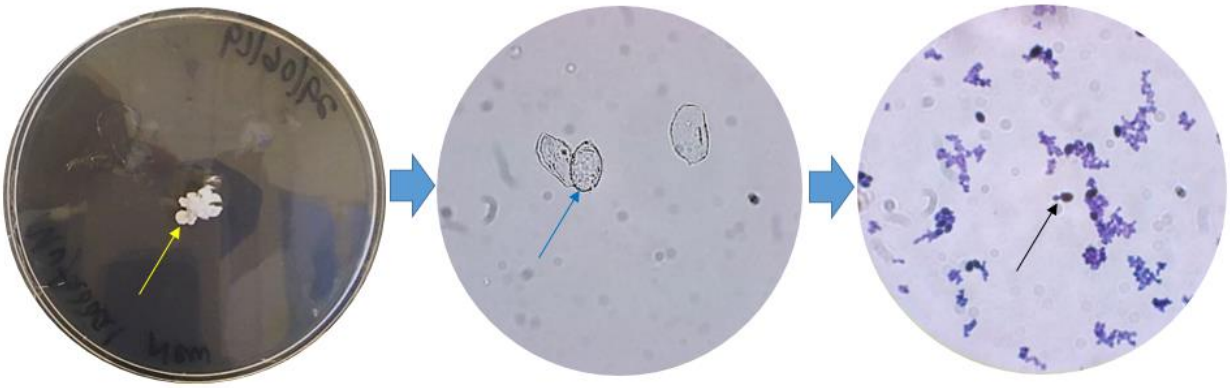

Fig. 2: *Phenotypic identification of the isolate* ***Legend: (A):*** *yeasty colonies appearance on SDA;* ***(B):*** *oval-shaped yeast in 20% KOH;* ***(C):*** *Gram-positive (purple) budding yeast.*

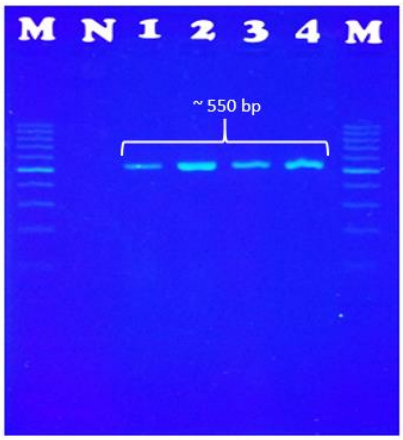

Fig. 3: *Agarose gel electrophoresis photograph of the PCR amplicons of the suspected isolate amplified using ITS1/4 universal primers.* ***Legend:*** *Lanes N: non-template control; Lanes 1 – 4: C. parapsilosis (positive isolate band size ~ 550 bp). Lanes M = DNA molecular weight marker 100 bp (ThermoFisher).*

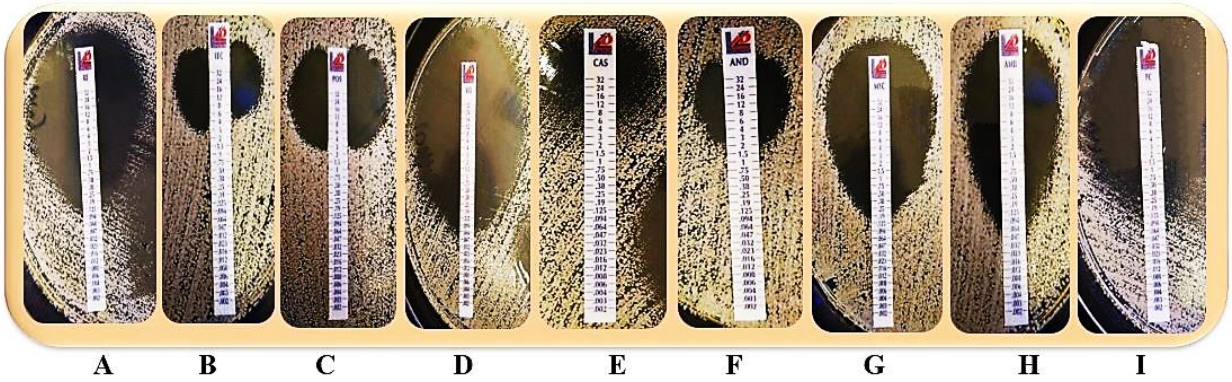

Fig. 4: *E-Test AFST results on the isolate:* ***A*** *= Ketoconazole (KE),* ***B*** *= Itraconazole (ITR),* ***C*** *= Posaconazole (POS),* ***D*** *= Voriconazole (VO),* ***E*** *= Caspofungin (CAS),* ***F*** *= Anidulafungin (AND),* ***G*** *= Micafungin (MYC),* ***H*** *= Amphotericin B (AMB) and* ***I*** *= Flucytosine.*

Table 1. The MICs (*μ*g/ml) of antifungal agents for *C. parapsilosis* clinical isolate determined by E-test after 24 h using CLSI standards.

Fungus	Antifungal agent	MIC (*μ*g/ml)
***Candida* Isolate**	Amphotericin B	0.25
	Fluconazole	>32
	Ketoconazole	>32
	Itraconazole	>32
	Voriconazole	0.125
C. parapsilosis	Posaconazole	>12
	Micafungin	0.16
	Anidulafungin	0.32
	Caspofungin	0.25

Key: *CLSI: Clinical and Laboratory Standards Institute, MIC: minimal inhibitory concentrations were determined after incubation for 24 h.*

DISCUSSION

Accurate identification of clinical isolate is paramount to speed up therapy and clinical judgment. Generally, *C. parapsilosis* was previously considered to be non-virulent when compared to *C. albicans.* Silva et al. [12] attributed the increased relevance of non-*albicans* species partly due to the advances in diagnostic methods, increased use of azoles, and the increased population of immunosuppressed patients. *Candida parapsilosis* is a non-obligate human pathogen, found in soil, insects, and sometimes human commensal organism. It is mostly isolated from human hands' subungual infection as reported by Trofa et al. [5]. The trend of AST of *C. parapsilosis* varies based on the strain tested. According to the findings by Won et al. [13], *C. parapsilosis* indicated susceptibility to micafungin (MIC 4 μg/ml) which is less sensitive compared to our isolate (0.16 μg/mL). However, the fluconazole tested against their isolates indicated decreased susceptibility to fluconazole (MIC ≥ 4 μg/ml) contrary to our findings which showed total resistance (MIC ≥ 32 μg/mL). This agrees with Choi et al. [10] that reported MIC ≥ 8 – 64 μg/mL from the isolates that had Y132F substitution in the *ERG11* gene. Another study conducted by Norimatsu et al. [11] on *C. parapsilosis* case isolates obtained from 80-year-old patients indicated susceptibility of the isolate to all the three classes of antifungals tested, with very low MIC values results including fluconazole (MIC 0.25 mg/L). The two tests' MIC agreed when the difference between the MIC endpoints was within two dilutions (±one dilution). The comparative evaluation of E-test and BMD CLSI reference MICs among the three tested antifungals, indicates good agreement *(r = 0.999)*, one dilution step higher in caspofungin than BMD.

Contrary to the findings of Arendrup et al. [12] that reported one dilution step lower in caspofungin compared to BMD. The MIC values agree in Amp B in both methods (Table 3). Simultaneously, for fluconazole, the agreement was two dilutions higher in E-test compared to BMD but still indicates the isolate's resistance ability (MIC ≥ 8) (Table 3). This agrees with the findings of Nedret et al. [13] that also reported fluconazole MIC discrepancies in both methods.

In conclusion, we report a case of *C. parapsilosis* pan-azole strain isolated from the hand of an immunocompetent person who has had a history of handling fungal isolates. We found that E-test showed the best performance for caspofungin

and Amp B in concordance with the BMD reference standard. The susceptibility of the isolate to caspofungin could be due to a rare prescription of the drug, perhaps due to its cost and daily intravenous mode of administration. The susceptibility observed in the isolate due to Amp B could be a result of its toxicity history and intravenous mode of administration. The resistance attributed to azoles may be due to their common prescription, less costly and oral administration availability. The Low susceptibility of the isolate or complete resistance to azole derivatives antifungals indicates that the isolate is an azole-resistant strain and the use of any of the tested echinocandins derivatives (micafungin, anidulafungin or caspofungin) will likely be helpful in the treatment as all of them indicated low MIC values against the isolate. Although the phenotypic methods are readily used in many laboratories, they could not offer to help in species identification. Molecular-based fungal identification using ITS sequence has become a necessity for speedy, sensitive and specific detection of the target isolate for better treatment outcomes and prevention of rising incidence of antifungal resistance.

CONCLUSION

The isolate may likely bear many variable resistance genes since it was resistant to each representative of the antifungal classes tested. Final identification and AFST of the isolate predicted and guided the therapy.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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عدوى خميرة فروة الرأس من غير البيض في صبي يبلغ من العمر 6 سنوات: تقرير حالة عن شلل المبيضات واختبار الحساسية لمضادات الفطريات

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المستخلص

الخلفية والاهداف. المبيضات البيضاء مسببة في معظم الالتهابات السطحية والأغشية البشرية ، على الرغم من أن الأنواع الأخرى مثل C. glabrataو C. Tropis و C. parapsilosis و C. kefyr و C. krusei و C. تشمل المواقع المصابة الأكثر شيوعًا الوجه والجلد وفروة الرأس واليدين. هدفت الدراسة إلى تحديد العامل المسبب وتحديد اختبار الحساسية لمضادات الفطريات (AFST) للعزلة المسؤولة عن عدوى فروة الرأس من أجل التقييم المناسب لاستجابة الإدارة. **دراسة حالة**. وصفنا حالة صبي يبلغ من العمر 6 سنوات ، مصاب بعدوى في فروة الرأس ناجمة عن داء المبيضات الشلل ، مع أعراض الحكة والجفاف والبقع المستديرة والثعلبة. لم ينجح العلاج التجريبي باستخدام 1٪ هيدروكورتيزون ثم تمت مراجعته لاحقًا مع محلول بيتاميثازون 0.1٪. تم علاج المريض بنجاح باستخدام مرهم موضعي يحتوي على 2٪ كيتوكونازول لمدة 8 أسابيع بعد التشخيص. **النتائج**. أظهرت العزلة نتائج متغيرة للتركيز المثبط الأدنى بين الأدوية المضادة للفطريات (أزول وإكينوكاندين) المختبرة. تُظهر العزلة قيم MIC عالية ≤ 3 و ≤ 4 ميكروغرام / مل إلى أحدث تريازول بوساكونازول وإيتراكونازول على التوالي ، على الرغم من أن بوساكونازول هو الجيل الثاني من تريازول. علاوة على ذلك ، في فئة echinocandins ، أظهر micafungin نتيجة MIC منخفضة (≤ 0.19 ميكروغرام / مل) ، ثم يليه2 ⩽) anidulafungin ميكروغرام / مل) وأظهر الكاسبوفنجين مقاومة (≤ 32 ميكروغرام / مل). أظهر أمفوتيريسين ب (بوليين) قيمة MIC منخفضة (≤ 0.64 ميكروغرام / مل) وأشار الفلوسيتوزين إلى قيمة MIC تبلغ 0.5 ميكروغرام / مل. أشار Ketoconazole و voriconazole إلى انخفاض0.125 ⩽) MIC ميكروغرام / مل و 0.64 ميكروغرام / مل على التوالي) وبالتالي ، وجه العلاج بسبب توافر الكيتوكونازول وفعاليته من حيث التكلفة. **الخاتمة**. تسلط هذه النتائج الضوء على أهمية التعرف على الملاحظة المتزايدة لسلالات C. parapsilosis في عدوى فروة الرأس. استجاب المريض للعلاج وبدأ نمو الشعر بعد الأسبوع السادس من العلاج وتعافى المريض تماما في نهاية العلاج.

الكلمات الدالة . Azoles، Candida parapsilosis، فروة الرأس ، اختبار الحساسية لمضادات الفطريات ، PCR، الاختبار الإلكتروني