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Candida albicans interdigital foot infection: A case report highlighting the importance of antifungal susceptibility testing

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***Candida* species are opportunistic fungal pathogens which are often isolated from immunocompromised individuals. *Candida albicans* is the most frequently isolated species in both superficial and invasive candidiasis. Non-*albicans* species are equally striving in their pathogenic ability, but it is noticed that *C. albicans* continues to exert its relevance as the leading cause of candidiasis. This is confirmed by this case report finding, from the traditional laboratory culture-based phenotypic methods to molecular diagnostic methods and finally DNA sequencing. Antifungal susceptibility pattern was performed using E-test strip to determine the minimum inhibitory concentrations (MICs) of eight antifungal agents from the three main classes against *C. albicans* isolate. The MIC results were read at 24 and 48 h incubation according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The results indicate susceptibility of *C. albicans* to amphotericin B with MIC value of 0.47 µg/mL, anidulafungin with MIC of 0.32 µg/mL; micafungin with MIC of 0.94 µg/mL and caspofungin with MIC of 0.125 µg/mL. The isolate was found to be resistant to all the four azole derivatives tested: fluconazole MIC ≥256 µg/mL; itraconazole, posaconazole and voriconazole with MIC values ≥32 µg/mL, indicating that the isolate may be azole resistant strain. Determination of the susceptibility pattern of this isolate is paramount for effective management of the case. Use of any echinocandins derivatives may be of help in the treatment of such fluconazole resistant strain. Here, we report a case of interdigital space infection (between 4th and 5th digits) due to *C. albicans* in a 41 year old African man.**

Key words: *Candida albicans*, E-test, interdigital, African, superficial.

INTRODUCTION

Very few *Candida* species namely, *Candida albicans*, *Candida glabrata*, *Candida dubliniensis* and *Candida*

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parapsilosis are successful colonizers of human hosts. *C. albicans* is the commonest opportunistic human pathogen, more than any other *Candida* species, residing in the epithelial surfaces; it is in optimum check by the immune system and protective bacterial microbiome (Ventolini et al., 2016). When the defense system of the host becomes compromised, candidal infection may occur, due to these infective species, which ranges from superficial to life-threatening invasive candidiasis. Significant rise in the candidal infection due to *C. albicans* continues to be noticed as a result of increase in the population of immunocompromised individuals and the advances in the diagnosis of infections (Wagner et al., 2018). The success of *C. albicans* in causing cutaneous candidiasis is based on its ability to thrive in many environmental factors by exploring many pathogenic mechanisms to facilitate its survival. These mechanisms include; yeast–to–hyphae transition; biofilm formation, sensing and thigmotropistic growth; presence of adhesion molecules; secretion of enzymes e.g. hydrolases and phenotypic switching among others. Other important factor is the genetic predisposition that makes the skin more susceptible to candida infection, e.g. autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), popularly known as polyglandular autoimmune syndrome type 1 (PAS-1) caused as a result of mutations in autoimmune regulator gene (*AIRE*) (Kämpe et al., 2018). There are many types of superficial candidal infections; common ones include erosio interdigitalis blastomycetica, intertrigo, diaper dermatitis, perianal dermatitis, and candidal balanitis with symptoms such as scaling, redness, itching, and pain due to pressure exertion. Here, we report a case of *C. albicans* isolated from the interdigital space between 4th and 5th digits of the right foot of an immunocompetent individual. Antifungal susceptibility testing is crucial in the selection of the antifungal therapy. Ketoconazole and fluconazole were used as topical and oral treatment respectively, prior to clinical diagnosis, though the infection is not yet resolved.

CASE REPORT

A 41 year old African man, with suspected fungal infection on his right foot between 4th and 5th digits, presented with symptoms of scaling, fissure, itching and pain due to pressure exertion because of the massive growth and scaling of the entire space. The infection throve for a long time from the onset of its symptoms till presentation due to favourable environment such as moisture and high relative humidity enjoyed by the aetiologic agent. These factors are attributed to his frequency in the use of cover shoes during laboratory hours that provides favourable condition of humidity for fungal growth and coupled with commonly available climatic conditions in his home country, where the infection sets in. The condition further worsened, since he came to Malaysia where higher rainfall of almost 10 months in a year is experienced. This suggests high relative humidity which is required by the infective agent and that led to the presentation for diagnosis. The importance of this report is to provide available data for researchers as well as provide identity of the aetiologic agent for the choice of appropriate treatment, since the important *Candida* species had the history of drug resistance. On day 0, the scales of the affected area were aseptically scraped, after cleaning the area with 70% alcohol and placed in 10% KOH to remove the debris of the epithelial cells and to release the infective organism from the compacted scales. The cells were cultured on Sabouraud dextrose agar (SDA) (Merck, Germany) and the process of identification continued up to day 13, where the direct DNA sequencing of the organism was done. The sequences obtained were subjected to BLAST software using the National Center for Biotechnology Information (NCBI) database for confirmation of the organism identity. The clinical tests conducted are sequentially described in Figure 1.

MATERIALS AND METHODS

Laboratory culture

The laboratory culture was carried according to the protocol of Wagner et al. (2018). The scaly sample scraped from the site was first dissolved in 10% KOH preparation to soften and release the yeast cells from the scaly sample. Then a direct microscopy using 40x objective for basic yeast features was made (data not shown) and inoculation was subsequently done on primary inoculation media, SDA and incubated overnight at 37°C as shown in Figure 2A. The pure isolate in plate B as indicated in Figure 2B was obtained by sub-culturing onto another sterile SDA plate and incubated in the same way as in plate A for 72 h.

Culture on CHROMagars candida

The suspected *Candida* species was cultured on commercially prepared plate of CHROMagar Candida media (Isolab, Malaysia), and incubated over night at 37°C for growth and colour appearance.

Gram staining technique

Gram staining was carried out by staining the dried and heat fixed light smeared colony using distilled water on a clean grease free slide; crystal violet was used the primary stain, Lugol's iodine as mordant, acid alcohol as decolorizer and safranin as secondary stain. It was washed briefly using distilled after each step as described by Boyanova (2018).

Germ tube test

The germ tube test was carried out by incubating the single colony

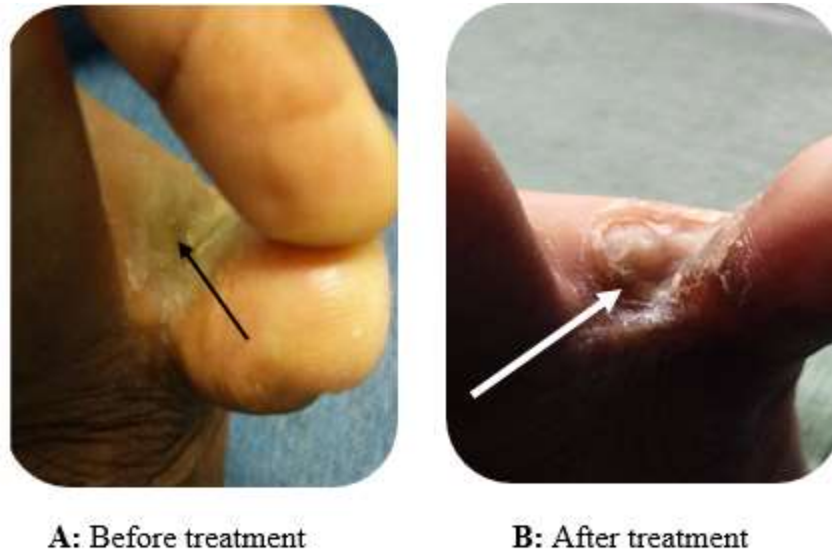

Figure 1 A and B. Clinical appearance of interdigital cleft infection between 4th and 5th digits with *C. albicans* before and after treatment with ketoconazole and fluconazole drugs (arrows: scales formed on the affected area).

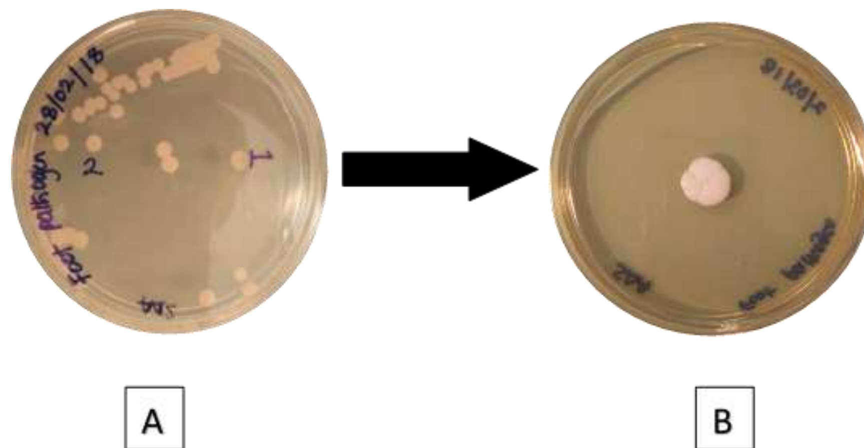

Figure 2. A) Inoculated SDA plate after scraping and treating of sample with 10% KOH. **B)** Pure clinical isolate of *C. albicans* grown at 37°C for 72 h on SDA media sub-cultured from plate A.

of the isolate in 3 ml of human serum at 37°C for 3 h as described by Jan et al. (2018).

PCR based identification method

The DNA was extracted using phenol-chloroform based method. The PCR amplification was run using BioRad Gradient PCR machine (U.S.A) and *Taq* DNA polymerase enzyme (Thermo scientific, U.S.A) together with ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers in a 25 µL volume of PCR reaction. The PCR reaction cycles were carried out in three basic steps (denaturation, annealing and extension). The initial denaturation occurred at 95°C, for 3 min, for 1 cycle, then followed by denaturation at 95°C, for 30 s and 30 cycles. After denaturation, then there was the annealing stage with temperature *(Ta)* of 55°C, for 30s and 30 cycles, followed by the extension stage at 72°C for 1 min using 30 cycles. At the end, the final extension occurred at 72°C for 5 min using 1 cycle.

Gel electrophoresis

The gel was prepared at 0.8% and the PCR products were loaded in their respective wells; it was submerged in the electrophoresis

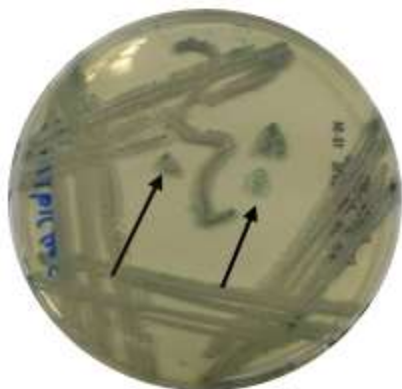

Figure 3. Suspected *C. albicans* pure isolate grown on CHROMagars Candida plate, with green colour appearance.

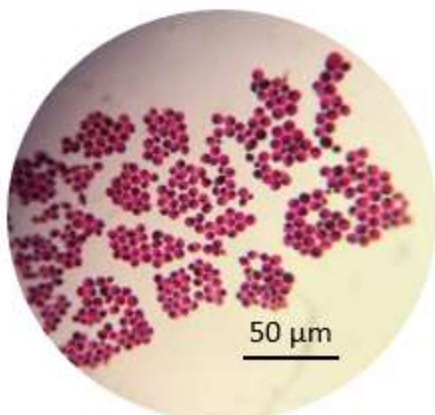

Figure 4. Gram stain reaction of yeast smear indicating Gram positive yeasts with purple colour appearance using 40x objective.

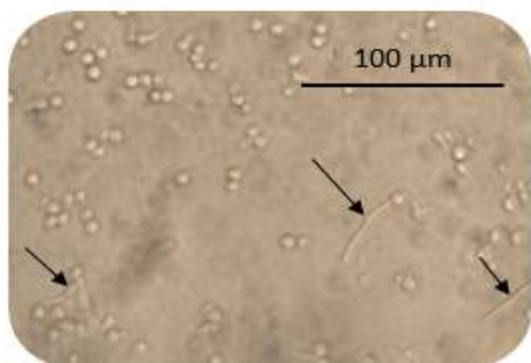

Figure 5. Result of germ tube produced by *C. albicans* (arrows: indicate germ tubes produced) using 40x objective.

tank containing 1x TBE buffer, using 5:1 ratio of sample to loading dye. The electrophoresis was set at 70V and 400 mA for 75 min.

DNA sequencing analysis

The PCR product obtained after carrying out the PCR reaction was purified using GeneAll® Expert purification kit (Seoul, Korea) and sent to 1st Base Scientific, Malaysia for direct DNA sequencing analysis.

Antifungal susceptibility testing using E – test strip

The E - test (Liofilchem, Italy) susceptibility testing was carried out according to the manufacturer's protocol using antifungal agents; amphotericin B, fluconazole, voriconazole, posaconazole, itraconazole, micafungin, anidulafungin and caspofungin test strips plated over Mueller Hinton Agar (MHA) supplemented with 2% glucose and 0.5 µg/mL methylene blue. The inoculum was adjusted to a final concentration of 0.5 to 2.5 x10^3 CFU/mL and swabbed on the dried MHA prepared plates and incubated at 35°C for 24 to 48 h for MIC determination of eclipse zone of inhibition.

RESULTS

Laboratory culture result

The isolate developed creamy to white yeasty and smooth surface colonies after 24 h incubation as shown in Figure 2A for initial culture and 2B for pure colony formation.

CHROMagars Candida result

The isolate appeared green in colour on the CHROMagar Candida culture plate as shown in Figure 3, suggesting that the isolate was either *C. albicans* or *C. dubliniensis*. Therefore, further analysis was required to unravel the two suspecting isolates.

Gram staining results

The yeast cells upon tested using Gram staining technique appeared purple, round to oval large budding cells indicating Gram positive reaction, a characteristic of yeast cells (Figure 4).

Germ tube result

The germ tube was produced and it appeared as a short outgrowth of hyphae, which is 3 – 4 times longer than its yeast origin as shown in Figure 5. This is suggestive that the isolate could be *C. albicans,* but the test is not sensitive to confirm it until further analysis is done. That led to the PCR based assay to confirm the causative isolate.

PCR gel electrophoresis result

Following the end of the electrophoresis separation, the gel was visualized under the UV light using AlphaImager® 2200 gel documentation system (Protein Simple, U.S.A). All the six sample wells (lanes 2 to 7) showed a band size of approximately ~ 600 bp and likewise the expected positive control of 943 bp (Lane 1) as shown in Figure 6.

DNA sequencing results

The following nucleotide sequence obtained from sequencing result was subjected to BLAST software using NCBI database and the causative agent was identified as *C. albicans* with 99% homology to NCBI GenBank database (Table 1).

DNA sequence of *C. albicans* obtained from sequencing analysis

DNA sequence of *C. albicans* obtained from sequencing analysis was:

5’AACATCCGCTACTGAGGCATCCCTGCTTGGTTTCTT
TTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTA
GTCCTACCTGATTTGAGGTCAAAGTTTGAAGATATAC
GTGGTGGACGTTACCGCCGCAAGCAATGTTTTTGGT
TAGACCTAAGCCATTGTCAAAGCGATCCCGCCTTAC
CACTACCGTCTTTCAAGCAAACCCAAGTCGTATTGCT
CAACACCAAACCCAGCGGTTTGAGGGAGAAACGACG
CTCAAACAGGCATGCCCTCCGGAATACCAGAGGGCG
CAATGTGCGTTCAAAGATTCGATGATTCACGAATATC
TGCAATTCATATTACGTATCGCATTTCGCTGCGTTCT
TCATCGATGCGAGAACCAAGAGATCCGTTGTTGAAA
GTTTTGACTATTAAGTAATAATCTGGTGTGACAAGTT
GATAAAAAATTGGTTGTAAGTTTAGACCTCTGGCGGC
AGGCTGGGCCCACCGCCAAAGCAAGTTTGTTTCAAA
GAAAAACACATGTGGTGCAATTAAGCAAATCAGTAAT
GATCCTTCCGCAGGTTCACCTACAGAAACCTTGTGTT
CAACGACTAAATAATT3’

Antifungal susceptibility testing results

Antifungal susceptibility testing was performed on the identified *C. albicans* isolate using E - test method (Liofilchem, Italy). The susceptibility pattern of the isolate was determined using published MIC breakpoint of CLSI (M27-A3) and literature guidelines for *in vitro* susceptibility testing of *C. albicans* (Table 2). Eight antifungal agents were tested on the isolate as indicated in Table 3. The MIC reading was determined where the relevant inhibition eclipse intersects the strip and the MIC was read and evaluated on the scale as indicated in Figure 7 by the same two investigators with a third investigator designated to resolve results discrepancies.

DISCUSSION

C. albicans is basically an asexual, polymorphic fungus that grows as either ovoid-shaped budding yeast or an elongated ellipsoidal yeast cells, with constriction at the septa (pseudohyphae). Its success as a pathogen lies on its genetic, biochemical, and morphological flexibility which facilitate its adaptation to a wide range of host niches. It is the most prevalent and best studied *Candida* species, and acts as both commensal and human pathogen as described by da Silva Dantas et al. (2016). Its change of status from commensal to pathogenic depends on the slight change of environmental conditions leading to expression of virulence factors like adhesins and invasins on its cell surface, production of hydrolases that mediate adhesion to susceptible host and biofilms formation as reported by Mayer et al. (2013). *C. albicans* is also associated with interdigital space infection and found to be conformed to the findings of Metin et al. (2018) which indicated that *C. albicans*, has a strong predilection for the intertriginous areas of the inguinal folds, intergluteal cleft, axillae, inframammary folds, umbilicus and the web spaces, particularly between the third, fourth, and fifth digits of the hands or feet.

The frequent isolation of *C. albicans* justifies its pathogenicity and is supported by the presence of underlying predisposing factors such as cancer, diabetes, prolonged use of antibiotics, hormonal therapy, oral contraception and pregnancy as reported by Metin et al. (2018). The global burden of superficial fungal infection (skin, hair and nail) was put to approximately one billion and is mostly associated with candida clade with *C. albicans* on the lead in causing mucosal disease, *Trichophyton* in skin diseases and *Aspergillus fumigatus* in allergic fungal disease (Bongomin et al., 2017). In Malaysia, according to the laboratory-based surveillance on *Candida* species between 2000 and 2013 conducted on hospitalized patients, with samples obtained from different anatomical sites, indicated fifteen *Candida* species. Occurrence of candidiasis on the skin of the lower extremities e.g. foot and hand, corroborated the findings of Mandengue and Denning (2018) that reported active penetration of yeast, also breakdown of the physical skin barrier as a contributing factor.

The molecular based technique of identifying the suspected aetiologic agent was based on amplification of target sequences in the internal transcribed spacer (ITS) regions of the noncoding ribosomal RNA operon as described by Okata-Karigane et al. (2018) using specific ITS 1 and 4 primers, because the region has the ability of discriminating or characterizing several *Candida* species based on species specific DNA polymorphism. In conclusion, here we report a case of *C. albicans* isolated

Table 1. DNA sequence analysis result of *C. albicans* when subjected to BLAST software using NCBI database.

Code used	Suspected genera	Identified species	Product length (bp)	Sequencing query length	Percentage of homology to NCBI GenBank database	NCBI accession number
Yeast	Candida	*Candida albicans*	~600	601	575/579 (99%)	KY101880.1

Table 2. CLSI (M60/M27-A2) and literature guidelines for *in vitro* susceptibility testing of *C. albicans.*

Antifungal agent	Interpretative criteria (µg/ml)			
	Susceptible (S)	Intermediate (I)	Susceptible dose-dependent (S - DD)	Resistant (R)
Amphotericin B	-	-	-	-
Fluconazole	≤2	-	4	≥8
Itraconazole	≤0.125	0.25 – 0.5	-	≥1.0
Voriconazole	≤0.125	0.25 – 0.5	-	≥1.0
Posaconazole	≤0.125	0.25 – 0.5	-	≥1.0
Micafungin	≤0.25	0.5	-	≥1.0
Anidulafungin	≤0.25	0.5	-	≥1.0
Caspofungin	≤0.25	0.5	-	≥1.0

CLSI, Clinical and Laboratory Standards Institute, MIC: minimal inhibitory concentration.

Table 3. MICs (µg/ml) of antifungal agents for *C. albicans* clinical isolate determined by E-test after 24 h incubation.

Candida isolate	Antifungal agent	MIC (µg/ml)/(Range)	Range references
C. albicans	Amphotericin B	0.47 (0.125 – 8)	Arendrup et al. (2017)
	Fluconazole	≥256 (0.125 – 0.25)	Fothergill et al. (2014)
	Itraconazole	≥ 32 (0.015 – 0.12.5)	Dadar et al. (2018)
	Voriconazole	≥32 (0.0313 – 4)	Siopi et al. (2015)
	Posaconazole	≥32 (0.03 – 8)	Badiee et al. (2017)
	Micafungin	0.94 (0.015 – 8)	Fothergill et al. (2014)
	Anidulafungin	0.32 (0.019 – 0.5)	Kidd et al. (2018)
	Caspofungin	0.125 (0.03 – 0.5)	Mahdavi Omran et al. (2018)

from the foot of an immunocompetent individual, in order to find the best treatment option. Determination of antifungal susceptibility pattern for *Candida* species is often important and necessary for clinical decision and better patient management, as a result of increase in frequency of resistant *Candida* species isolation in many infections. Larkin et al. (2018) reported high MIC value of fluconazole antifungal in resistant *C. albicans*. The resistance to azole antifungals may be due to resistance to fluconazole. According to Eldesouky et al. (2017), there is strong positive correlation (R = 0.9) between the MIC of fluconazole (MIC ≥16 µg/ml) and the MICs (≥2 µg/ml) of posaconazole, itraconazole, voriconazole and ravuconazole, suggesting cross resistance. This especially occurs in *C. glabrata* and agrees with our findings where all the four azole derivatives tested indicate resistance with high MIC values (≥32 µg/ml).

Treatment

Though no any clinical test was conducted then to determine the identity of the aetiologic agent, oral (fluconazole) and topical (ketoconazole) antifungal agents were used for 1 to 4 weeks. Such treatment only subdued the infection for a while, without completely curing the mycosis. After the diagnosis, same ketoconazole was also prescribed by the examining

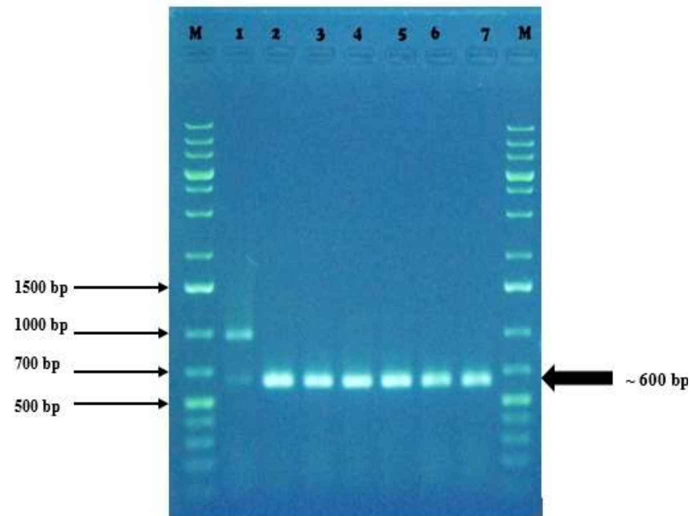

Figure 6. Gel result of the separated *C. albicans* internal transcribed spacer amplified DNA. Lanes M = 1 kb plus DNA ladder bands; lane 1= positive control (PC: 943 bp); lanes 2 to 7 showed positive isolate bands (side arrow: ~ 600 bp).

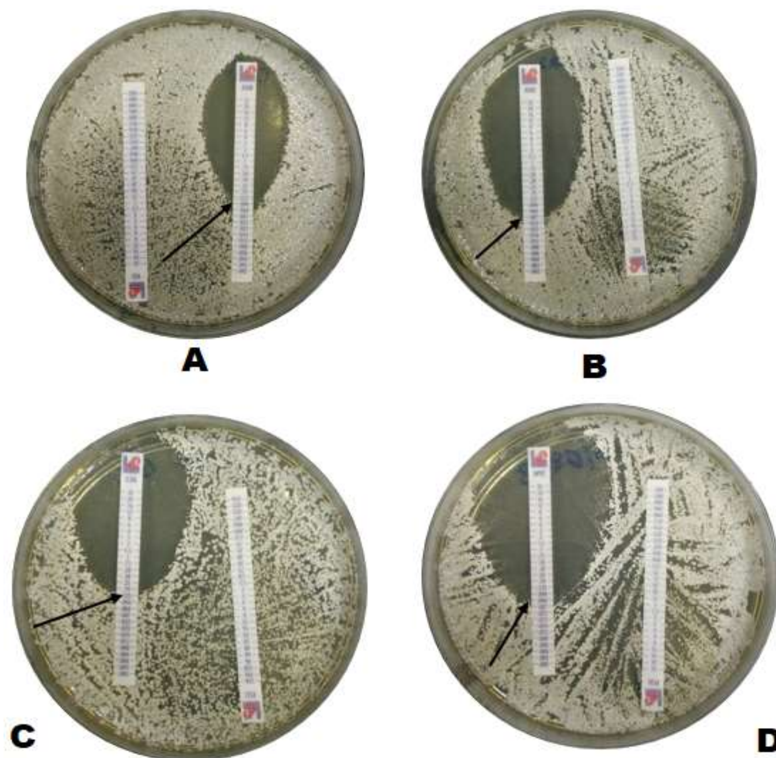

Figure 7. A. *C. albicans* susceptible to amphotericin B antifungal (MIC = 0.47µg/mL) (right left E-test strip); *C. albicans* resistant to voriconazole antifungal (right strip). **B.** From right: *C. albicans* susceptible to anidulafungin antifungal (MIC = 0.32 µg/mL); from left: *C. albicans* resistant to itraconazole antifungal. **C.** From right: *C. albicans* susceptible to caspofungin antifungal (MIC = 0.25µg/mL); from left: *C. albicans* resistant to fluconazole antifungal. **D.** From right: *C. albicans* susceptible to micafungin antifungal (MIC = 0.94µg/mL); from left: *C. albicans* resistant to posaconazole antifungal.

doctor and it is yet to yield any positive response.

Conclusion

The susceptibility testing results confirmed the reason for treatment failure, using fluconazole and ketoconazole before diagnosis of the isolate. Non-susceptibility of the isolate to four azole antifungals tested indicates resistance and therefore azole antifungal is not an option for the treatment of such case. In contrast, the low MIC values of anidulafungin, micafungin and caspofungin within the range of 0.125 to 1.0 µg/mL show promising option on the use of echinocandins for the treatment of fluconazole resistant *C. albicans*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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