RESEARCH ARTICLE

Isolation, Characterization and Molecular Identification of Multidrug Resistance from *Candida* Species from Various Clinical Samples

Shruthi Pirsingla[1*], Lakshmi Krishnasamy[2], Koka Sri Sandhya[3]

[1]*Asst professor, Department of Microbiology, Prathima Institute of Medical Sciences, Nagnoor, Karimnagar, India.*
[2]*Professor, Department of Microbiology, Sree Balaji Medical college and hospital, Chennai TN, India.*
[3]*Professor and HOD, Department of Microbiology, Prathima Institute of Medical Sciences, Nagnoor, Karimnagar, India.*

Received: 02nd August, 2024; Revised: 10th August, 2024; Accepted: 18th August, 2024; Available Online: 31st August, 2024

ABSTRACT

Background and Purpose: The most prevalent fungus pathogens in humans are said to be *Candida* species. *Candida* pathogens have become more common in the last several years. But in the absence of any risk factors, these infections are uncommon. To that end, the current study set out to identify the species of *Candida* responsible for various clinical samples and establish the risk factors for candiduria.

Materials and Methods: The 100 samples from patients who were suspected of having an aging, diabetes, prolonged hospital stays, organ transplantation, recurring bacterial infections, antibiotic use and catheter use were used in the current investigation. Of them, 100 instances involved candidiasis out of which 20 are MDR. Using the germ tube test, colony staining on CHROMagar medium, intracellular beta-glucosidase enzyme activity, and glucose absorption pattern, several species of *Candida* were identified. Afterwards, internal transcribed spacer region DNA sequencing verified the colonies with the same morphology.

Results: Based on the findings, *Candida albicans, C. glabrata, C. tropicalis*, and *C. krusei* were identified as 40, 32, 18, and 10% of the isolates, respectively. Diabetes, bacterial infections, and urinary tract infections were the most common predisposing factors (23%); however, it was proven in all patients that one or more other predisposing variables existed. Patients with these serious infections can only be treated with a restricted number of antifungal agents from a small number of drug classes. Resistance can be intrinsic or acquired; as a result, acquired resistance either develops in response to antifungal selection pressure in the particular patient or, less frequently, results from the horizontal transmission of resistant strains between patients. Multidrug resistance is 20%, but more and more reports of it have been observed in several *Candida* species, most notably *C. glabrata* and more recently *C. auris*. The factors that contribute to multidrug resistance include overall antifungal use (fluconazole and amphotericin B), subtherapeutic drug levels at infection/colonization sites, drug sequestration in the biofilm matrix, and, in the event of outbreaks, suboptimal infection control.

Conclusion: The most common fungal species, according to the results, was *C. albicans*. Furthermore, bacterial infections were more common. We go over the molecular causes of resistance, treatment and preventative methods.

Keywords: Multidrug resistance, Antifungal, CHROMagar, *Candida albicans*, *Candida glabrata*.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.83

How to cite this article: Pirsingla S, Krishnasamy L, Sandhya KS. Isolation, Characterization and Molecular Identification of Multidrug Resistance from *Candida* Species from Various Clinical Samples. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1646-1649.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

There are two types of candiduria: asymptomatic and symptomatic. The majority of individuals with urinary *Candida* excretion are asymptomatic. Conversely, individuals with renal candidiasis, pyelonephritis, cystitis, epididymorchitis, and prostatitis exhibit symptomatic candiduria.[1] Fungal infections that are deep can cause candiduria. Research indicates that the majority of cases where there is a documented growth of *Candida* in urine cultures are temporary and unrelated to systemic illness.[2] Nonetheless, candiduria develops in 10% of blood infections brought on by *Candida*.[3] *Candida* yeast is known to induce renal and urinary tract infections, which are characterized by discomfort, dysuria, micturating, hematuria, and pyuria.[2] Usually do these infections happen in the absence of any risk factors. Accordingly, a number of significant risk factors for these infections include aging, diabetes, prolonged hospital stays, organ transplantation, recurring bacterial infections, antibiotic use, and catheter use. Catheter use is the most common risk factor for candiduria, particularly in patients hospitalized to intensive care units.[4]

**Author for Correspondence: shruthipirsingla@gmail.com*

In this regard, a study on UTI found that 26.5% of catheter users experienced UTIs brought on by *Candida* species.[5] In order to cause irritation to the mucosa, catheters offer a surface on which organisms can adhere and colonize into the bladder.[6] C. albicans is the primary pathogen identified from the majority of clinical samples among the species of *Candida*.[7] However, *C. glabrata* was presented as the dominating species in an international monitoring survey.[8] *Candida tropicalis, parapsilosis, krusei, guilliermondii*, *kefir*, and other species have also been isolated from urinary tract infections.[9,10] The epidemiology, risk factors, and species distribution of candiduria have all been studied in a few small-scale research. It appears that the difficulty of differentiating between the colonization of infections makes the definition of candiduria challenging.[1] In individuals with candiduria, recurrent urine cultures and imaging tests to identify the infection foci are useful methods for establishing a differential diagnosis.[11] Because different species of *Candida* have different patterns of susceptibility to antifungals, it is vital to identify them, even though the death rate from candiduria cases is low.[12, 13] For instance, an infection caused by *C. glabrata* treated as a result of fluconazole medication.[14] For the illness to be properly treated and to avoid becoming chronic, the microbial agent responsible for the infection must be identified. However, other research indicates that managing UTIs by the elimination of risk factors may be more successful than treating them.[14, 15] The current study's objectives were to ascertain the incidence rate of candiduria and the part that predisposing factors played in isolation and management of this infection.

MATERIAL AND METHODS

The study was carried out between September 2023- August 2024 in the Department of Microbiology, Prathima Medical College and Hospital, Nagnoor, Karimnagar, Telangana State. After obtaining Ethical clearance. A total of 100 consecutive *Candida* isolates from various clinical specimens like a high vaginal swab, midstream clean-catch urine, Catheterized urine, blood, sputum, pus, and skin scraping from patients with candidiasis were included in the study.

Inclusion criteria

The study includes *Candida* isolates from various clinical samples sent routinely to the Microbiology laboratory

Exclusion criteria

Patients receiving any kind of antifungal medication six weeks prior to sample collection are excluded. Conventional Mycology techniques were employed to isolate *Candida* spp. from these specimens. The direct specimen was used for gram staining, and the specimens were injected into Sabouraud's dextrose agar slants and incubated for 24 hours at 37°C. Following a germ tube test, *C. albicans* or *C. dubliniensis* were found to be the positives. Chlamydospore development on cornmeal agar and growth at 45°C were additional methods used to identify *C. albicans*. For the ultimate confirmation of species, a sugar fermentation and assimilation test were performed on each of the 100 isolates. In accordance with the manufacturer's instructions, the *Candida* spp. were simultaneously inoculated on CHROM agar and incubated at 37°C for 24 hours. The species were identified by the type and color of the colonies on CHROM agar media. (Table 1)

Table 1: Color various Candiadias sps on CHROM agar for identification

S. No	*Name*	*Color on CHROM Agar*
1	*C. albicans*	Light green
2	*C. tropicalis*	Blue with pink halo
3	*C. krusei*	Pink
4	*C. glabrata*	Pink to Purple
5	*C. parapsilosis*	Cream to pale pink

MDR Mechanism

The majority of MDR *Candida* infections involve isolates from species that have intrinsic resistance, such as *C. glabrata* and *C. krusei*, which are resistant to echinocandin,[16-19] or infections with *C. guilliermondii* or *C. auris*, which are intrinsically resistant to multiple drugs and are currently spreading throughout India and other continents, as previously mentioned. It is uncommon for animals lacking intrinsic resistance to develop multidrug resistance since doing so usually necessitates the development of several resistance mechanisms, which can be costly in terms of fitness.[20, 21] However, in *C. dubliniensis* and *C. albicans*, ERG3 and ERG2 changes have been linked, separately, to azole and amphotericin B cross-resistance.[22, 20, 22, 23] Furthermore, isolates of *Candida* carrying ERG11 mutations have been shown to exhibit azole and polyene resistance in conjunction with either ERG3 or ERG5 changes have been shown to be resistant to azole and polyene.[22, 24] Lastly, XDR has only been reported in a very small number of patients receiving alternating, long-term antifungal medication. A patient with mucosal infection developed azole, echinocandin, and amphotericin B resistance in C. albicans over a 1-year period.[25] Similarly, *C. glabrata* developed azole, flucytosine, and echinocandin resistance as a result of mutations in FUR1 (CgFUR1) and CgFKS2, as well as overexpression of CgCDR1 and CgCDR2 during 20 weeks of antifungal therapy in a recipient of hematopoietic stem cell transplant.[26]

DISCUSSION

Treatment options for MDR *Candida* infections are still limited, and expert guidelines lack evidence to support their recommendations.[27, 28] Anecdotal experience indicates that a higher clinical failure rate occurs in patients with *C. glabrata* infections that have elevated MICs to the echinocandins and fluconazole resistance as well (MDR strains); in these patients, liposomal amphotericin B combined with a thorough search for undrained or unremoved foci of infection (like central catheters, abdominal abscesses, other hardware, or thrombophlebitis) is essential to a positive outcome.[29] Although there is a lack of clinical link between experimental animal data with certain less important FKS mutations and higher response rates with higher doses of medication in people with

MDR C. albican s infection, those results are suggestive.[30, 31] Limited experience is available to guide the best course of treatment for patients with MDR *C. auris*; however, susceptibility data indicate that echinocandin resistance is less common than that of the azoles, meaning that, when it comes to clinically available antifungal agents, echinocandin or amphotericin B remain the likely drugs of choice for those infections.[11, 5, 32] Antifungal susceptibility testing is highly advised in certain circumstances, and a thorough mycological assessment for underlying resistance mechanisms for isolates with increased MICs may be appropriate. It is impossible to draw firm conclusions on antifungal agent combinations due to the paucity of data on these combinations. For those patients, more recent treatments from different medication classes are required to enhance outcomes.

It is essential to implement prevention techniques to stop the spread of MDR and XDR diseases. Effective source control for infections connected to the abdomen and devices will, as was previously said, lessen the infection load and eradicate persister cells, which have the potential to develop medication tolerance over time.[33] Furthermore, hardware and gadgets need to be removed in order to get rid of biofilms, which can be a drug-protected infection source. Antifungal medication shouldn't be used at subtherapeutic levels as this can promote the development of resistance, and treatment shouldn't be kept up for longer than necessary. In established high-risk individuals, prophylactic treatment of infection is necessary to prevent infection; nevertheless, a meticulous effort should be made to establish a mycological diagnosis of infection so that susceptibility testing can aid in the management of antifungals.

CONCLUSION

Pharmacological resistance to antifungals, including MDR the species *Candida* has grown in significance in the treatment of invasive fungal illnesses. These infections have a high rate of morbidity and mortality and may spread via healthcare settings. In many medical centers, MDR *C. glabrata* has grown increasingly prevalent and poses serious management issues. Comparably, *C. auris* has become a significant clonally spreading species that exhibits MDR and XDR traits as well as long-term colonization and widespread environmental pollution. Since these resistant infections have poor outcomes, management should be optimized with a precise mycological diagnosis and therapy directed by susceptibility testing. To help patients with these infections, newer and advanced therapies are required. The accurate species identification of *Candida* is important for the treatment because not all species respond to the same treatment and also because of the increasing antifungal resistance. CHROMagar is a convenient and rapid method of identification of *Candida* species, especially in resource-limited poor settings.

REFERENCES

1. Revankar SG, Kirkpatrick WR, McAtee RK, et al. Detection and significance of fluconazole resistance in oropharyngeal candidiasis in human immunodeficiency virus-infected patients. J Infect Dis 1996; 174:821–7. 7.
2. Redding S, Smith J, Farinacci G, et al. Resistance of *Candida albicans* to fluconazole during treatment of oropharyngeal candidiasis in a patient with AIDS: documentation by in vitro susceptibility testing and DNA subtype analysis. Clin Infect Dis 1994; 18:240–2.
3. Marr KA, Lyons CN, Rustad TR, Bowden RA, White TC, Rustad T. Rapid, transient fluconazole resistance in *Candida albicans* is associated with increased mRNA levels of CDR. Antimicrob Agents Chemother 1998; 42:2584–9. 9.
4. Lewis JS 2nd, Wiederhold NP, Wickes BL, Patterson TF, Jorgensen JH. Rapid emergence of echinocandin resistance in *Candida* glabrata resulting in clinical and microbiologic failure. Antimicrob Agents Chemother 2013; 57:4559–61.
5. Vallabhaneni S, Cleveland AA, Farley MM, et al. Epidemiology and risk factors for echinocandin no susceptible *Candida* glabrata bloodstream infections: data from a large multisite population-based candidemia surveillance program, 2008–2014. Open Forum Infect Dis 2015; 2:ofv163.
6. Redding SW, Marr KA, Kirkpatrick WR, Coco BJ, Patterson TF. *Candida* glabrata sepsis secondary to oral colonization in bone marrow transplantation. Med Mycol 2004; 42:479–81.
7. Pappas PG, Kauffman CA, Andes DR, et al. Executive summary: clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016; 62:409–17.
8. Moran GP, Sullivan DJ, Henman MC, et al. Antifungal drug susceptibilities of oral *Candida* dubliniensis isolates from human immunodeficiency virus (HIV)- infected and non-HIV-infected subjects and generation of stable fluconazole-resistant derivatives in vitro. Antimicrob Agents Chemother 1997; 41:617–23.
9. Tumbarello M, Sanguinetti M, Trecarichi EM, et al. Fungaemia caused by *Candida* glabrata with reduced susceptibility to fluconazole due to altered gene expression: risk factors, antifungal treatment and outcome. J Antimicrob Chemother 2008; 62:1379–85.
10. Garcia-Effron G, Katiyar SK, Park S, Edlind TD, Perlin DS. A naturally occurring proline-to-alanine amino acid change in Fks1p in *Candida* parapsilosis, *Candida* orthopsilosis, and *Candida* metapsilosis accounts for reduced echinocandin susceptibility. Antimicrob Agents Chemother 2008; 52:2305–12.
11. Lockhart SR, Etienne KA, Vallabhaneni S, et al. Simultaneous emergence of multidrug-resistant *Candida* auris on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis 2017; 64:134–40.
12. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW; European Committee on Antimicrobial Susceptibility Testing–Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST). EUCAST technical note on *Candida* and micafungin, anidulafungin and fluconazole. Mycoses 2014; 57:377–9.
13. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts; 4th informational supplement. Document M27-S4. Wayne, PA: CLSI, 2012.
14. Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. Increase in *Candida* krusei infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N Engl J Med 1991; 325:1274–7.
15. Westbrook SD, Kirkpatrick WR, Freytes CO, et al. *Candida* krusei sepsis secondary to oral colonization in a hemopoietic stem cell transplant recipient. Med Mycol 2007; 45:187–90
16. Alexander BD, Johnson MD, Pfeiffer CD, et al. Increasing

echinocandin resistance in *Candida* glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 2013; 56:1724–32
17. Jensen RH, Justesen US, Rewes A, Perlin DS, Arendrup MC. Echinocandin failure case due to a previously unreported FKS1 mutation in *Candida* krusei. Antimicrob Agents Chemother 2014; 58:3550–2.
18. Bordallo-Cardona MA, Escribano P, de la Pedrosa EG, et al. In vitro exposure to increasing micafungin concentrations easily promotes echinocandin resistance in *Candida* glabrata isolates. Antimicrob Agents Chemother 2017; 61. pii:e01542-16.
19. Forastiero A, Garcia-Gil V, Rivero-Menendez O, et al. Rapid development of *Candida* krusei echinocandin resistance during caspofungin therapy. Antimicrob Agents Chemother 2015; 59:6975–82.
20. Vincent BM, Lancaster AK, Scherz-Shouval R, Whitesell L, Lindquist S. Fitness trade-offs restrict the evolution of resistance to amphotericin B. PLoS Biol 2013; 11:e1001692.
21. Ben-Ami R, Kontoyiannis DP. Resistance to echinocandins comes at a cost: the impact of FKS1 hotspot mutations on *Candida albicans* fitness and virulence. Virulence 2012; 3:95–7.
22. Martel CM, Parker JE, Bader O, et al. Identification and characterization of four azole-resistant erg3 mutants of *Candida albicans*. Antimicrob Agents Chemother 2010; 54:4527–33.
23. Morio F, Pagniez F, Lacroix C, Miegeville M, Le Pape P. Amino acid substitutions in the *Candida albicans* sterol Δ5,6-desaturase (Erg3p) confer azole resistance: characterization of two novel mutants with impaired virulence. J Antimicrob Chemother 2012; 67:2131–8.
24. Pinjon E, Moran GP, Jackson CJ, et al. Molecular mechanisms of itraconazole resistance in *Candida* dubliniensis. Antimicrob Agents Chemother 2003; 47:2424–37.
25. Martel CM, Parker JE, Bader O, et al. A clinical isolate of *Candida albicans* with mutations in ERG11 (encoding sterol 14alpha-demethylase) and ERG5 (encoding C22 desaturase) is cross resistant to azoles and amphotericin B. Antimicrob Agents Chemother 2010; 54:3578–83
26. Chapeland-Leclerc F, Hennequin C, Papon N, et al. Acquisition of flucytosine, azole, and caspofungin resistance in *Candida* glabrata bloodstream isolates serially obtained from a hematopoietic stem cell transplant recipient. Antimicrob Agents Chemother 2010; 54:1360
27. Pappas PG, Kauffman CA, Andes DR, et al. Executive summary: clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 2016; 62:409–17.
28. Cornely OA, Bassetti M, Calandra T, et al.; ESCMID Fungal Infection Study Group. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect 2012; 18:19–37.
29. Alexander BD, Johnson MD, Pfeiffer CD, et al. Increasing echinocandin resistance in *Candida* glabrata: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. Clin Infect Dis 2013; 56:1724–32.
30. Perlin DS. Echinocandin resistance in *Candida*. Clin Infect Dis 2015; 61: S612–7.
31. Wiederhold NP, Najvar LK, Bocanegra RA, Kirkpatrick WR, Patterson TF. Caspofungin dose escalation for invasive candidiasis due to resistant *Candida albicans*. Antimicrob Agents Chemother 2011; 55:3254–60.
32. Chowdhary A, Voss A, Meis JF. Multidrug-resistant *Candida* auris: 'new kid on the block' in hospital-associated infections? J Hosp Infect 2016; 94:209–12.
33. Kathuria S, Singh PK, Sharma C, et al. Multidrug-resistant *Candida* auris misidentified as *Candida* haemulonii: characterization by matrix-assisted laser desorption ionization-time of flight mass spectrometry and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI broth microdilution, and Etest method. J Clin Microbiol 2015; 53:1823–30.