ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 6, June 2021

Isolation, speciation and antifungal susceptibility testing of vaginal swab

[1] Dr Sendilkumar, [2]Dr Sangeetha.S,[3] Dr Lakshminarayana S.A , [4]Dr Santhoshini Vaijinath
[1][2][3][4] Department of Microbiology,Rajarajeswari Medical College & Hospital, Bengaluru, Karnataka, India.

Abstract: -

Background:

Candida species is a normal commensal flora of human body inhabiting skin, mucous membranes and gastrointestinal tract but may be associated with superficial and deep-seated fungal infections.1,2 Candida species are responsible for various clinical infections ranging from mucocutaneous infection to life threatening invasive diseases along with increased resistance to antifungal drugs has made a serious concern. Resistance to antifungal agents has increased during the last decade. Thus, identification of Candida up to species level and its antifungal susceptibility testing has a significance in the management of Candida infections.3,4
Aim of the study is to isolate, characterize & to determine the anti-fungal susceptibility pattern of all Candida speciesisolatedfrom vaginal swab.

Methods:

A total of 45 Candida species were isolated from 748 vaginal swab over 12 months period. Growthon Sabouraud dextrose agar were evaluated for colony appearance, macroscopic examination, Gram staining, germ tube test and urea hydrolysis test. Further, they were processed for Candida speciation on CHROMagar. Antifungal susceptibility testing was performed as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A document.5

Results:

Among 45 patients with symptoms, 33.33% (15) where pregnant women with Candida vaginitis gave positive cultures from high vaginal swabs. The major species were C. albicans 60% (27), C. glabrata 22.3% (10), C. tropicalis17.7% (8). Among the Candida species (non albicans), (10) Candida glabrata was 22.3% and (8) Candida tropicalis was 17.7%. Antifungal susceptibility tests shows 100% resistance to fluconazole by the Candida species (non albicans) such as (10) Candida glabrata and 47.5% to (8) Candida tropicalis. 64% resistance to clotrimazole,74% resistance to Fluconazole, 62 % resistance to Ketoconazole and 55% resistance to Voriconazole.100% sensitive to amphotericin B and nystatin by all Candida species.

Conclusions:

Candida albicans was the predominant species responsible for Candidal vaginitis & is resistant to most commonly used antifungal drugs clotrimazole, ketoconazole and fluconazole. The increasing number of resistances to antifungal agents' cases among Candida species demands for Isolation, speciation and antifungal susceptibility testing of Candidal vaginitis as well as other fungal infections

INTRODUCTION

Candida species are one of the major causes of Candidal vaginitis worldwide ranking 7th most common 6.

Phylum	Ascomycota
Class	Hemiascomycetes
Order	Saccharomycetales
Family	Candidacea
Genus	Candida 7,8

Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics and antifungal susceptibility. All species of Candida have been shown to cause a similar spectrum of disease ranging from mucosal disease like oral thrush to invasive disease, however difference in disease severity and susceptibility to different antifungal agents have been reported5. Despite the availability of an expanded antifungal armamentarium the mortality associated with invasive Candida infection remains high ranging 19% to 49%.9,10

VIRULENCE FACTORS

The state of the host is of primary importance in determining Candida pathogenicity. However, there are factors associated with the organism, which contributes to its ability to cause disease and explain the differences among species in their pathogenicity. The most relevant virulence factors of Candida species are11.12

- Toxins

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 6, June 2021

- Complement receptors
- Adhesion
- Phenotypic switching
- Enzyme production

PATHOGENESIS OF CANDIDIASIS

Candidiasis is mostly an endogenous infection arising from overgrowth of the fungus inhabiting normal flora. The gastrointestinal tract is considered a major reservoir for Candida from which the fungus can invade the blood stream following damage to the GI mucosa causing deep seated/disseminated infection. It is believed that Candida can cross the intact GI mucosa by a process called persorption following fungal overgrowth due to excessive antibiotic treatment.13,14

However, it may be occasionally acquired from exogenous sources (such as catheters or prosthetic devices). This is of particular importance in the development of deep seated and systemic candidiasis as most of these therapeutic modalities are used in compromised hosts whose defence system are unable to combat the introduced pathogen.15

Once Candida enters the blood stream whether from exogenous or endogenous source, the microorganisms adhere to the endothelial surface before dissemination into tissues. Person to person transmission may be occasionally seen in cases such as oral candidiasis in neonates of mothers with vaginal candidiasis or endophthalmitis following corneal transplantation from an infected donor.16,17,18

Vaginal candidiasis is observed most frequently in pregnant and diabetic women. The predisposing factors associated with the hosts include pregnancy, oral contraceptives, prior antibiotic therapy, immunological and nutritional factors. Besides Candida albicans, Candida glabrataand Candida tropicalis are the most frequently isolated Candida. 19,20

MATERIALS AND METHODS

The study was conducted in the Microbiology department of Rajarajeswari Medical College & Hospital over a period of one year from January 2019 to December 2019. A total of 748 vaginal swabs were included in the study during this period. The study was reviewed and approved by the Institutional Ethical Committee RRMCH

Processing: Direct examination of all vaginal swab samples was done, wet film & Gram stain was prepared and observed for budding yeast cells and pseudo hyphae. Primary culture was done on blood Agar. Once the colonies morphologically resembling Candida were observed on primary plates, Gram-stained smear examination was performed. The colonies on blood Agar were sub cultured on to two SDA plates, incubated one at room temperature and the other at 370C to get a pure growth. Plate was incubated at 28^0C for 48 hours. After 48 hours, the areas where the cuts were made in the media, were examined first under the low power objective and then under the high-power objective for the presence of 21,22

1. Hyphae - true or pseudo hyphae
2. Blastoconidia
3. Arthroconidia
4. Chlamydospores

- The isolated colonies were stained by Gram's stain and then the confirmed yeast colonies were selected for further processing like germ tube test, corn meal agar, Hichrome candida differential agar, sugar assimilation and fermentation tests.

Antifungal Susceptibility testing:

- Antifungal susceptibility test for the Candida isolates was done by Disk Diffusion method. The inoculum suspension was prepared by picking five colonies of 1mm diameter from a 24-hour old culture and suspended in 5ml sterile 0.85% NaCl. The turbidity of the cell suspension was adjusted to 0.5 McFarlands' turbidity standard.23

Disk Diffusion Method.

Medium:

- Mueller Hinton Agar supplemented with 2% dextrose and 0.5µg/ml methylene blue was used. The following antifungal discs were used fluconazole 25µg, amphotericin B 100 U, ketaconazole10µg, voriconazole 1µg, Clotrimazole10µg and nystatin 100U. The plates were inverted and incubated at 35^0C within 15 minutes after the disks were applied. Plates were examined after 20 to 24 hours of incubation. In case of insufficient growth, plates were read at 48 hours. The resulting zone of inhibition was measured to the nearest whole millimetre at the point where there was prominent reduction in growth.24,25

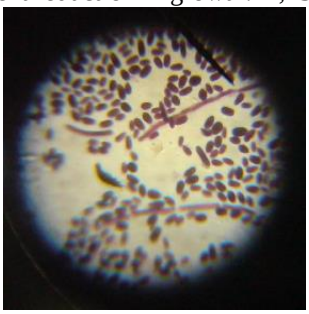

Fig.1- Gramstain of Candida

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 6, June 2021

Fig. 2 – Candida on SDA

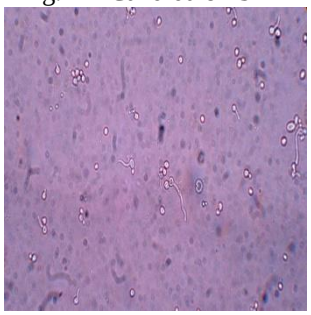

Fig. 3 - Germ Tube (Candidaalbicans)

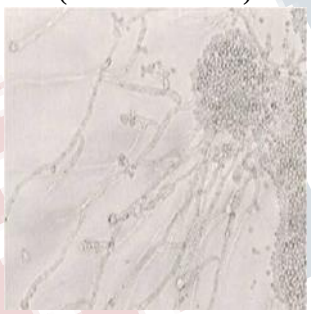

Fig. 4 - Chlamydospore of Candida albicans

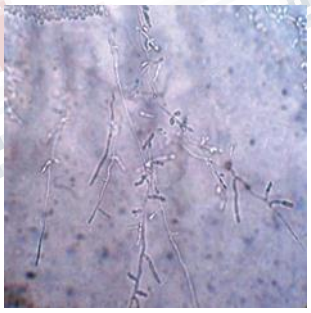

Fig. 5 - C.tropicalis (microscopy on cornmeal agar)

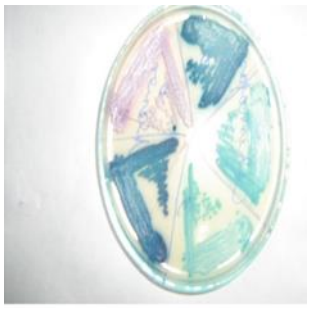

FIG 6: Candida on HiCrome agar

FIG 7:Sugar assimilation test

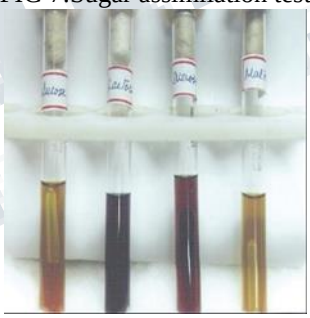

Fig 8:Sugar fermentation test

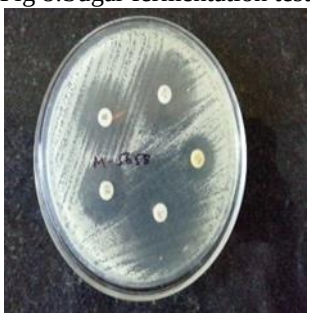

FIG 9:Anti-fungal susceptibility test

RESULTS

The present study was carried over 748 clinical samples which yielded Candida. The age of the patients ranged from 17 to 90 years female.

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 6, Issue 6, June 2021

Table – 1: Age wise distribution of Candida isolates

AGE (in Years)	TOTAL(PATIENTS)
0-30	7
31-60	22
61-90	16
TOTAL	**45**

Majority of the patients where Candida was isolated were from the age group of 31- 60 years 48.8% (22), followed by the age group of 31-60 years 35.5% (16) . The least Candida species isolated was in the age group of 0-30 years 15.5% (7) cases.

Table – 6: Distribution of Candida Species

SL.NO	SPECIES	TOTAL	%
1	*C. albicans*	27	60
2	*C. glabrata*	10	22.3
3	*C. tropicalis*	8	17.7
	TOTAL	**45**	**100%**

Table – 9: Antifungal susceptibility pattern of Candida species

Species(n)	CC n(%)	Flu n(%)	Ke n(%)	VO n(%)	Am-B n(%)	Nys n(%)
C. albicans(27)	21 (77.7)	19 (70.3)	25 (92.5)	26 (92.5)	27 (100.0)	27 (100.0)
C. glabrata(10)	6 (60.0)	0 (0)	7 (87.5)	9 (90.0)	10 (100.0)	10 (100.0)
C. tropicalis (8)	7 (87.5)	5 (62.5)	6 (75)	8 (100.0)	8 (100.0)	8 (100.0)
Total (45)	**34 (75.5)**	**24 (53.3)**	**38 (84.4)**	**43 (95.5)**	**45(100.0)**	**45(100.0)**

CC =Clotrimazole; Am-B = Amphotericin B, VO = Voriconazole Ke= Ketoconazole; Nys=Nystatin Flu = Fluconazole

DISCUSSION

Candida infections are one of the commonest nosocomial infections. Candida albicans has been the most frequently isolated species from clinical specimens, but now species of Candida appear to be increasing which have high azole resistance and are refractory to treatment. Vulvovaginal candidiasis is caused by overabundant growth of yeast cells, belonging to the Candida species, in the vaginal mucosa. Candida albicans infection occurs in the vast majority (80% to 90%).3 This highlights the importance of identifying Candida species within vaginal swabs in order to provide the physician/gynaecologist information concerning the proper treatment to their patients. This study has been done to correlate the clinical picture relation to candida species isolated along with their anti-fungal susceptibility.26,27

SUMMARY

In the present study vaginal swabs with were collected and processed in the Department of Microbiology, RRMCH, Bangalore, during the period January 2019 to December 2019. In this study 45 isolates were identified, various species of Candida on the basis of their morphological and biochemical reactions. Anti-fungal Susceptibility tests were performed for all the isolates of Candida.

The species of Candida were identified by Germ-tube test, Chlamydospore formation on corn meal agar and colony identification on Chrom Agar. The confirmation tests for species typing and other categorization was done by sugar fermentation test which in turn was compared to the reactions of sugar assimilation test.

A total of 748 vaginal swabs were received in 1 year, 45 isolates of Candida were identified 27 Candida albicans constituted for 60% and other Candida species constituted 40% of total isolates obtained.

- Among the Candida species (non albicans), 10 Candida glabrata was 22.3% and 8 Candida tropicalis was 17.7%
- Antifungal susceptibility tests show 100% resistance to fluconazole by the Candida species (non albicans) such as (10) Candida glabrata and 47.5% to (8) Candida tropicalis
- 64% resistance to clotrimazole,74% resistance to Fluconazole, 62 % resistance to Ketoconazole and 55% resistance to Voriconazole
- 100% sensitive to amphotericin B and nystatin by all Candida species.

• Hence, Candida infections require culture and identification and its correlation with clinical manifestations, development of antifungal resistance among different species emphasizes the need for the routine susceptibility testing for Candida.

CONCLUSION

In the present study, there is a clear that resistance to antifungal agents is ranging from 50-100 % apart from intrinsically resistant candida. The increasing number of resistances to antifungal agents' cases among Candida species demands for Isolation, speciation and antifungal susceptibility testing of Candidal vaginitis as well as other fungal infections.

The key to successful management lies in early recognizing high risk cases by diagnosing species with the aid of Hi-Chrome agar which takes only 24 to 48 hrs for speciation, as it serves as a comfortable alternative to conventional methods.

Among antifungal drugs in our study, Voriconazole is a better alternative to the other azoles for the treatment. Amphotericin-B can be used as a reserved drug for non-responding Candida infections.

Preventive measures against hospital infections are crucial in acquiring in the best policy. A high standard of hand hygiene and hygienic techniques to be followed and should be trained. Pharmacological interventional strategies should be emphasized including prudent use of antibiotics.

REFERENCES

1. Mohanty S, Xess I, Hasan F, Kapil A, Mittal S,Tolosa JE. Prevalence &susceptibility to fluconazole of Candida Species causing vulvovaginitis. Indian J Med Res 2007; 126:216–19.
2. Mohanty S, Xess I, Hasan F, Kapil A, Mittal S, Tolosa JE. Prevalence & susceptibility to fluconazole of Candida species causing vulvovaginitis. Indian J Med Res. 2007 Sep;126(3):216-9. PMID: 18037716.
3. Baron EJ, Cassell GH, Duffy LB, Eschenbach JR, Greenwood SM, Harvey NE, et al. Laboratory diagnosis of female genital tract infections. In: Baron EJ, editor. Cumulative techniques and procedures in clinical microbiology (Cumitech) 17A. Washington, DC: ASM Press; 1993. pp. 1–28. [Google Scholar]
4. Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS. Candida species: current epidemiology, pathogenicity, bioflm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013;62:10–24.
5. Gullo A. Invasive fungal infections: the challenge continues. Drugs. 2009;69(Suppl 1):65–73.
6. Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Tanabe K, Niimi M, Gofeau A, Monk BC. Efux mediated antifungal drug resistance. Clin Microbiol Rev. 2009;22:291–321.
7. ElFeky DS, Gohar NM, El-Seidi EA, Ezzat MM, AboElew SH. Species identifcation and antifungal susceptibility pattern of Candida isolates in cases of vulvovaginal candidiasis. Alex Med J. 2016;52:269–77.
8. Jayalakshmi L, RatnaKumari G, Samson SH. Isolation, speciation and antifungal susceptibility testing of candida from clinical specimens at a tertiary care hospital. Sch J App Med Sci. 2014;2:3193–8.
9. Jha BJ, Dey S, Tamang MD, Joshy ME, Shivananda PG, Brahmadatan KN. Characterization of Candida species isolated from cases of lower respiratory tract infection. Kathmandu Univ Med J. 2006;4:290–4.
10. Sajjan AC, Mahalakshmi VV, Hajare V. Prevalence and antifungal susceptibility of Candida species isolated from patients attending tertiary care hospital. IOSR J Dent Med Sci. 2014;13:44–9.
11. Achkar JM, Fries BC. Candidal infections of the genitourinary tract. Clin Microbiol Rev. 2010;23:253–73.
12. CLS Institute. Method for antifungal disk difusion susceptibility testing of yeasts: approved standard M44-A. Clinical and Laboratory Standards Institute: Wayne; 2006 (Material and Method).
13. Nolte FS, Parkinson T, Falconer DJ, Dix S, Williams J, Gilmore C, et al. Isolation and Characterization of fluconazole and amphotericin B-resistant Candida albicans from Blood of Two Patients with Leukemia. Antimicrob Agents Chemother1997; 44(1):196-99.
14. NCCLS. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guideline. NCCLS document M44-A. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.
15. Badiee, P., Badali, H., Boekhout, T. et al. Antifungal susceptibility testing of Candida species isolated from the immunocompromised patients admitted to ten university hospitals in Iran: comparison of colonizing and infecting isolates. BMC Infect Dis 17, 727 (2017). https://doi.org/10.1186/s12879-017-2825-7
16. Yang YL, Cheng HH, Ho YA, Hsiao CF, Lo HJ. Fluconazole resistance rate of Candida species from diferent regions and hospital types in Taiwan. J Microbiol Immunol Infect. 2003;36:187–91.
17. Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Tanabe K, Niimi M, Gofeau A,

Monk BC. Efux mediated antifungal drug resistance. Clin Microbiol Rev. 2009;22:291–321.

18. White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev. 1998;11:382–402.
19. Hospenthal DR, Beckius ML, Floyd KL, Horvath LL, Murray CK. Presumptive identifcation of Candida species other than C. albicans, C. krusei, and C. tropicalis with the chromogenic medium CHROMagar Candida. Ann Clin MicrobiolAntimicrob. 2006;5:1.
20. Isenberg HD. Mycology and Antifungal Susceptibility Testing. In: Gracia LS, Isenberg HD, editors. Clinical microbiology procedure handbook, vol. 2. 2nd ed. Washington, DC: ASM Press; 2004. p. 8.0.1–8.10.7.
21. Kaufman C, Fisher J. Candida urinary tract infections: diagnosis. Clin Infect Dis. 2011;52(suppl 6):S452–6.
22. Yucesoy M, Esen N, Yulung N. Use of chromogenic agar for the identifcation of Candida albicans strains. Kobe J Med Sci. 2001;47:161–7.
23. Murray CK, Beckius ML, Green JA, Hospenthal DR. Use of chromogenic medium for the isolation of yeasts from clinical specimens. J Med Microbiol. 2005;54:981–5.
24. Lee SC, Fung CP, Lee N, See LC, Huang JS, Tsai CJ, Chen KS, Shieh WB. Fluconazole disk difusion test with methylene blue- and glucose-enriched Mueller–Hinton agar for determining susceptibility of Candida species. J Clin Microbiol. 2001;39:1615–7.
25. Pfaller MA, Boyken L, Messer SA, Hollis RJ, Diekema DJ. Stability of Mueller–Hinton agar supplemented with glucose and methylene blue for disk difusion testing of fuconazole and voriconazole. J Clin Microbiol. 2004;42:1288–9.
26. Manikandan C, Amsath A. Characterization and susceptibility pattern of Candida species isolated from urine sample in pattukkottai, Tamilnadu, India. Int J Pure Appl Zool. 2015;3:17–23.
27. Mondal S, Mondal A, Pal N, Banerjee P, Kumar S, Bhargava D. Species distribution and in vitro antifungal susceptibility patterns of Candida. J Inst Med. 2013;35:45–9.