Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL01, ISSUE 04, 2010

Isolation, Identification and Speciation of Candida in various Clinical Samples

Suresh Babu[1]

[1]Associate Professor in Department of Microbiology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, Telangana, India.

Shilpa Lingala[2]

[2]Assistant Professor, Department of Microbiology, Fathima Institute of Medical Sciences, Kadapa, AP, India.

Corresponding Author: Suresh Babu[1]

ABSTRACT

Background: 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 paramount significance in the management of Candida infections. The aim of the study was to speciate Candida species and to determine antifungal susceptibility pattern of Candida species to antifungal agents.

Materials and methods: This is a prospective study conducted in the Department of Microbiology Tertiary Care Teaching Hospital over a period of 1 year. A total of 100 consecutive and non-repetitive Candida isolates from various clinical specimens like high vaginal swab, urine, sputum, pus, catheter tip, ear swab and stool sample from patients with antibiotic associated diarrhoea were included in the study. Gram's stain was performed from direct samples and inoculated on Sabouraud dextrose agar, incubated at 37°C for 24 hours. The isolates diagnosed to be fungus other than Candida species were excepted from the study. Colony on Sabouraud dextrose agar was processed for identification of Candida species. The isolates were subjected to macroscopic examination, Gram's staining, germ tube test and urea hydrolysis test.

Result: A total of 70 Candida spp. was isolated from various clinical samples. Distribution of samples of Candida isolates were mentioned in Table 2. Candida albicans (48.6%) was the most common species isolated. Among the non-albicans Candida, C. tropicalis (38%), C. krusei (22%), C. glabarata (10%) and C. dubliniensis (1%). Sensitivity and specificity of CHROM agar was 100% for C. tropicalis and C. krusei. Sensitivity and specificity for C. albicans was 100% and 94% respectively. Sensitivity and specificity for C. glabarata was 75% and 100%, for C. dubliniensis was 96% and 100%.

ISSN:0975 -3583,0976-2833 VOL01, ISSUE 04, 2010

Conclusion: Characterization of Candida to species level helps in identifying the intrinsically resistant species. Along with *Candida albicans*, non-albicans *candida spp* like *C. tropicalis, C. krusei*, *C. glabarata*, *C. dubliniensis* are increasingly being isolated from clinical specimens. CHROM agar is a simple, rapid and inexpensive method with good sensitivity and specificity for identification of such species.

Keywords: Candida, Non-albicans candida (NAC), Fluconazole, Amphotericin-B.

INTRODUCTION

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] The switch of Candida species from commensal to a potent pathogen is facilitated by various virulence factors such as adherence to host tissues, medical devices, biofilm formation, and secretion of extracellular hydrolytic enzymes. [2] Also, in recent year non-albicans Candida (NAC) species are considered as major pathogens causing severe infections in human. [3]

The commonly used antifungal drugs show significant variation in the susceptibility pattern among the types of Candida species. The drug resistance scenario has been increasing during last decades due to over growing use of random antifungal agents. [4] Several previous studies reported the emergence of drug resistance Candida species in global scenario [5]. Therefore, the change in drug susceptibility pattern of Candida species and introduction of newer antifungal agents has made the in vitro susceptibility testing of antifungal agents more relevant for using specific and sensitive drugs.

Thus, the isolation, identification, characterization and susceptibility testing of Candida species in clinical specimens have become increasingly important for management of fungal infections.

In the present study, we explored the characterization of Candida species using agar and showed the susceptibility pattern of Candida isolates from clinical specimens.

MATERIALS AND METHODS

This is a prospective study conducted in the Department of Microbiology Tertiary Care Teaching Hospital over a period of 1 year. A total of 100 consecutive and non-repetitive Candida isolates from various clinical specimens like high vaginal swab, urine, sputum, pus, catheter tip, ear swab and stool sample from patients with antibiotic associated diarrhoea were included in the study.

ISSN:0975 -3583,0976-2833 VOL01, ISSUE 04, 2010

Gram's stain was performed from direct samples and inoculated on Sabouraud dextrose agar, incubated at 37°C for 24 hours. The isolates diagnosed to be fungus other than Candida species were excepted from the study. Colony on Sabouraud dextrose agar was processed for identification of Candida species. The isolates were subjected to macroscopic examination, Gram's staining, germ tube test and urea hydrolysis test.

The creamy, pasty and yeasty colony that showed Gram positive budding yeast like cells with pseudohyphae and negative urea hydrolysis test were processed further. Germ tube test was performed, and germ tube positives were identified as either C. albicans or C. dubliniensis. Further identification of C. albicans was done by chlamydospore formation on cornmeal agar and growth at 45°C. All the isolates were subjected to sugar fermentation and assimilation tests for final confirmation of Candida species.

Simultaneously the Candida spp. were inoculated on CHROMagar (Hi-media, India) and incubated at 37°C for 24 hours and the species were identified by type and colour of the colonies on CHROMagar media as per manufacturer's instruction. (Figure 1 and Table 1). CHROMagar is a novel, differential culture medium that is claimed to facilitate the isolation by colorimetric presumptive identification.3

RESULTS

Table 1: Colour of various Candida spp. on CHROM agar for identification.10

Name	**Colour on CHROM agar**
C. albicans	Light green
C. tropicalis	Metallic blue
C. krusei	Rose pink
C. glabrata	White
C. parapsilosis	Pale cream
C. dubliniensis	Dark green

Table 2: Isolation of Candida spp. from clinical samples.

Sample	***C. albicans***	***C. tropicalis***	***C. krusei***	***C. glabrata***	***C. dubliniensis***	***No. of Candida isolates***
Vaginal swab	20	3	2	2	0	27
Urine	5	3	2	0	1	11
Sputum	3	9	3	1	0	16
Ear swab	3	2	0	2	0	7
Stool	2	2	0	0	0	4
Pus	0	0	4	0	0	4

ISSN:0975 -3583,0976-2833 VOL01, ISSUE 04, 2010

Catheter tip	1	0	0	0	0	1
Total	34	19	11	5	1	70

A total of 70 Candida spp. was isolated from various clinical samples. Distribution of samples of Candida isolates were mentioned in Table 2. Candida albicans (48.6%) was the most common species isolated.

Table 3: Sensitivity and specificity of CHROM agar for speciation of Candida.

Candida spp	No. of Candida spp. identified by conventional method	No. of Candida spp. identified using CHROM agar	Sensitivity of CHROM agar	Specificity of CHROM agar
C. albicans	35	34	100%	94%
C. tropicalis	19	19	100%	100%
C. krusei	11	11	100%	100%
C. glabarata	5	5	75%	100%
C. dubliniensis	0	1	96%	100%

Among the non-albicans Candida, C. tropicalis (38%), C. krusei (22%), C. glabarata (10%) and C. dubliniensis (1%). Sensitivity and specificity of CHROM agar was 100% for C. tropicalis and C. krusei. Sensitivity and specificity for C. albicans was 100% and 94% respectively. Sensitivity and specificity for C. glabarata was 75% and 100%, for C. dubliniensis was 96% and 100% (Table 3).

DISCUSSION

Candida spp. belongs to the commensal flora of human body and colonizes the mucosal surfaces soon after birth. There is an increasing incidence of candida infections among hospitalized patients mostly the immune-suppressed, and both host factors and virulence factors of candida spp. contribute to this. There are several host factors that predispose to candida infections like prolong use of Cortico steroids or antibiotics, poor nutrition, metabolic derangements and invasive procedures. Due to prolong and extensive use of anti-fungal drugs, there is a change in infection prevalence caused by different candida spp. and it is now important to identify the infective fungi up to species level as different species of candida vary in their virulence and anti-fungal susceptibility. [7]

Candida albicans is predominate species in our study. Studies done by different other authors like Manjunath et al. and Jayalakshmi L. has similar findings where Candida albicans is the predominate species. [8]

The incidence of non albicans candida (NAC) isolates is on rise and similar results have seen in studies done by various authors. [9] In year 2002, a study done by Kaviarasan et al. showed

ISSN:0975 -3583,0976-2833 VOL01, ISSUE 04, 2010

prevalence of NAC isolates was 39.5% which rise to 73.6% in year 2011 as shown in study by R Adhikary. [10] Empirical antifungal therapy depends on the species involved due to higher resistance rates to multiple antifungal agents in non albicans candida (NAC) isolates. This is also evident in our study where the resistance to most of the Azole antifungal agents is much higher in NAC isolates in compare to Candida albicans isolates. The higher resistance rates in NAC isolates have also been seen in studies done by Hii, Sabhapandit et al. and other authors. [11]

Since the inception of anti-fungal susceptibility testing by disc diffusion methods by CLSI in 2003 many studies by different authors showed the development of anti-fungal resistance in Candida spp. [12] The rise in resistance among NAC isolates for Fluconazole which is a frequently used Azole anti-fungal is evident from previous studies done by various authors. In year 2007, study by MA Pfaller. showed resistance rate to Fluconazole was 9.9% which rise in consecutive years the resistance rate rise as high as 32.4% as shown in a study done by Jayalakshmi L et al in year 2014. Our present study shows resistance rate of 60% which may be due to smaller number of isolates but go with the trend of increasing resistance rates. [13]

In our study C. glabrata was most predominate species (40%) among NAC isolates which contrasts from studies done by Sabhapanditet al. and Jayalakshmi L et al. in which C. tropicalis was the predominant species and C. glabrata was in second position. C. glabrata has emerged as an important opportunistic pathogen in last few decades and this is evident by study done by Li et al. and Trick. showing an extraordinary increase in its incidence. [14] Most of the Candida isolates were isolated from blood and urine samples (53%) which is similar to study done by Jayalakshmi L et al. and Sharma M. in which 42% and 43.3% of samples were blood and urine. [15] Most of the isolates (48%) in our study were from patients above 50 years of age and study done by Bhattacharjee et al. also show similar age group contributing major portion (44%) of the isolates. [16]

CONCLUSION

The present study shows that Candida spp. are an important opportunistic pathogen in ICU settings and patients from older age group is particularly vulnerable. The emergence of NAC isolates and the increasing resistance among NAC isolates to multiple anti-fungal drugs is a matter of concern to initiate empirical antifungal therapy. It is now important to identify the isolates to species level so that proper antifungal to which the isolate isn't intrinsically resistant can be initiated as empirical treatment. Constant monitoring of the changing epidemiology and resistance pattern of Candida species is needed to guide the clinicians and make proper antimicrobial policy.

ISSN:0975 -3583,0976-2833 VOL01, ISSUE 04, 2010

REFERENCES

1. Messer SA, Jones RN, Fritsche TR. International surveillance of Candida spp. and Aspergillus spp.: report from the SENTRY antimicrobial surveillance program (2003). J ClinMicrobiol. 2006; 44(5):1782–7.
2. Mokaddas EM, Al-Sweith NA, Khan ZU. Species distribution and antifungal susceptibility of Candida bloodstream isolates in Kuwait: a 10-years study. J Med Microbiol. 2007;56:255-9.
3. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004; 39(3):309-17.
4. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidaemia in Swiss tertiary care hospitals: secular trends, 1991-2000. Clin Infect Dis. 2004; 38(3):311-20.
5. Yang YL, Cheng HH, Ho YA, Hsiao CF, Lo HJ. Fluconazole resistance rate of Candida species from different regions and hospital types in Taiwan. J Microbiol Immunol Infect. 2003;36:187–91.
6. Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Tanabe K, Niimi M, Goffeau A, Monk BC. Efflux mediated antifungal drug resistance. Clin Microbiol Rev. 2009;22:291–321.
7. Hospenthal DR, Beckius ML, Floyd KL, Horvath LL, Murray CK. Presumptive identification of Candida species other than C. albicans, C. krusei, and C. tropicalis with the chromogenic medium CHROMagar Candida. Ann Clin Microbiol Antimicrob. 2006;5:1.
8. 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.
9. Anaissie EJ, McGinnis MR, Pfaller MA, eds. Clinical mycology. 2nd edn. Philadelphia: Churchill Livingstone 2009.
10. Mokaddas EM, Al-Sweih NA, Khan ZU. Species distribution and antifungal susceptibility of Candida bloodstream isolates in Kuwait: a 10-years study. J Med Microbiol 2007;56(Pt 2):255-259.
11. Moran, G.P., Sullivan, D.J., Coleman, D. C. *Emergence of non-Candida albicans Candida species as pathogens. In: Calderone RA. Candida and Candidiasis.*4, (2002).
12. Odds FC, B. R. CHROM agar Candida, a new differential isolation medium for presumptive identification of clinically important Candida spp. *J. Clin. Microbiol.*32, 1923–1929 (1994).
13. Pinjon, E., Sullivan, D., Salkin, I., Shanley, D. and Coleman, D. Simple, inexpensive, reliable method for differentiation of Candida dubliniensis from Candida albicans. *J. Clin. Microbiol.*36, 2093–2095 (1998).

ISSN:0975 -3583,0976-2833 VOL01, ISSUE 04, 2010

14. Shaheen MA, T. M. Species identification of Candida isolates from oral lesions of hospitalized and non hospitalized patients with oral candidiasis. *Egypt. Derm online Journal*,2, 1–13 (2006).
15. Odds, F.C., Bernaerts, R. 1994. CHROM agar Candida, a new differential isolation medium for presumptive identification of clinically important Candida spp. J. Clin. Microbiol., 32(8): 1923–9.
16. Maria Fátima Sugizaki, Cristianne Roberta Rhoden, Denise Mara Bombonatti, 1998. Prevalence and in vitro antifungal susceptibility of Candida spp. isolated from clinical specimens in São Paulo, Brazil. Rev Iberoam Micol., 15: 16–18.