Section: Microbiology

Original Research Article

ANTIFUNGAL SUSCEPTIBILITY PATTERN OF VARIOUS CANDIDA SPP ISOLATED IN A TERTIARY CARE CENTRE IN CENTRAL KERALA – A COMPARISON BETWEEN VITEK 2 SYSTEM AND E TEST

Meghana Venunath[1], Anitha Madhavan[2]

[1]Assistant Professor, Department of Microbiology, Travancore Medical College, Kollam, Kerala, India
[2]Associate Professor, Department of Microbiology, Govt. T.D. Medical College, Alappuzha, Kerala, India

Received : 08/11/2023
Received in revised form : 31/12/2023
Accepted : 16/01/2024

Keywords:
Candida, antifungal susceptibility, fluconazole, voriconazole, amphotericin B.

Corresponding Author:
Dr. Anitha Madhavan
Email: dranithaanoop2002@gmail.com

DOI: 10.47009/jamp.2024.6.1.246

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2024; 6 (1); 1239-1242

Abstract

Background: Infections due to Candida spp. are increasing especially in immunocompromised and critical care unit patients. Antifungal susceptibility tests are important to optimize antifungal treatment considering the increasing rates of Candida non albicans species and emergence of acquired antifungal resistance in some Candida species. This study was aimed at characterization of Candida spp. isolated from various specimens using VITEK2 system with special emphasis on isolates obtained from critical care areas and comparison of the antifungal susceptibility pattern of isolated Candida spp. using VITEK 2 system and E-test. **Materials and Methods:** 60 clinical isolates of Candida spp. were speciated using conventional tests and VITEK 2 automated system . Antifungal susceptibility automated method, the Vitek 2 system (VK2), was compared to E test procedure for fluconazole, voriconazole and Amphotericin B susceptibility. **Result:** Candida non albicans species predominated the isolates. Candida non albicans showed only low-level resistance to azoles and all Candida albicans isolates were azole sensitive. For azoles, essential agreement ranged from 95% to 100% and 100% for amphotericin B. **Conclusion:** The AST-YS01 Vitek 2 card system (bioMérieux) is a reliable standardized automated antifungal susceptibility test and showed comparable results to E test and thus may be used alternately.

INTRODUCTION

Candida species are the leading cause of invasive fungal infections in hospitalized patients and are the fourth most common isolates recovered from cases of nosocomial bloodstream infections.[1] Candidemia is associated with mortality of about 30-40%.[2]

An increase in incidence of Candida infections have been reported recently due to risk factors like widespread use of broad spectrum antibiotics, exposure to invasive procedures, underlying immunodeficiency states and malignancies.[3] Due to the increasing size of the patients at special risk (neutropenia, immunosuppression, metabolic dysfunction), Candida spp continues to be threat in the critical care patients with increasing number of Non-albicans Candida (NAC) being isolated. A majority of infections are commonly attributed to five species—C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, and C. krusei.[4] C. guilliermondii and C. lusitaniae are slowly emerging as new causative agents of invasive candidiasis. While C glabrata and C. parapsilosis were being reported from North America and Europe, recent reports have established them as emerging pathogens in the critical care patients from India too.[5] Recent evidence suggests that the majority of infections produced by this pathogen are associated with biofilm growth.[6] Speciating the Candida isolates is very critical in the management of these diseases as different species vary in their susceptibilities to the antifungals tested in vitro.[7] Non-albicans Candida demonstrate innate or acquired resistance to fluconazole, the most cost-effective and readily available antifungal drug for treatment of candidiasis.[8] Secondary resistance to amphotericin B has been described in C. tropicalis, C. parapsilosis, C. lusitaniae, and C. haemulonii.[9] Thus Antifungal susceptibility testing permits an accurate treatment selection and can significantly contribute to the understanding of local and global fungal resistance epidemiology.[10]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

The VITEK 2 system (bioMérieux, Inc.,) permits both species identification and antifungal susceptibility testing within 18 h compared to 48–72 h for the other methods. The mean time-to-result for the VITEK 2 system is 15 hours for amphotericin B (with a range of 11 to 27.8 hours) and fluconazole (with a range of 9 to 24.2 hours) and 12.4 hours for voriconazole (with a range of 8.1 to 25.1 hours).[11]

E-test is a recently commercialized simple and rapid method of antimicrobial susceptibility testing. After incubation for 24-48 hours for the diffusion of the antifungal agent, the oval-shaped inhibition zone of candidal growth indicate the minimal inhibitory concentrations (MICs).[12]

Since the susceptibility patterns of Candida spp. are usually unknown, empirical therapy is usually provided to the patient. Hence it is important to find out the various prevalent species in various regions due to their varied resistance profile against commonly used antifungal drugs. It not only reduces the cost of patient care by providing appropriate antifungal agent but also helps in contributing to the local fungal resistance epidemiology data. Commercially available E-strips and automated systems like VITEK2 systems provide an easier and cost effective alternative which can also significantly reduce the turn around time of the specimens in a busy clinical laboratory.

MATERIALS AND METHODS

This Cross-sectional Study was conducted in the Department of microbiology, Government T.D. Government Medical College, Alappuzha, India from January-December 2019. The study samples included all the isolates of candida spp obtained in the Department from various sterile sites (blood, urine, sputum, Endotracheal aspirates, Pus Aspirates, central line tips, other body fluids).

Candida spp. isolated was identified preliminarily by gram staining, germ tube test and pigment production in chrome agar. The isolates were then subcultured on SDA slants to obtain pure cultures and to ensure viablility. The identification was confirmed by VITEK2 systems. Subsequently, antifungal susceptibility testing was performed for Fluconazole, Voriconazole and Amphotericin B simultaneously using VITEK2 system and E test.

E-strips of Fluconazole (MIC range of 0.016-256 µg/ml), Voriconazole (MIC Range of 0.002-32 µg/ml), and Amphotericin B (MIC Range of 0.002-32 µg/ml) was used. RPMI 1640 broth with 2% glucose and 1.5% Bacto agar, buffered to pH 7.0 with 0.165 N-morpholino propanesulfonic acid (MOPS) buffer was used for testing. Statistical analysis was performed using SPSS 16.00 software.

RESULTS

A total of 60 isolates obtained from various sterile sites – blood(6), pus (24) and urine (30) were included in the study. VITEK final identification of "excellent," "very good," "good," was considered to be acceptable identification. Candida non albicans was the predominant species isolated from 44(77.33%) samples whereas Candida albicans was isolated from 16 (26.6%) samples. Candida non albicans spp isolated were C. parapsilosis (23,38.33%), C.tropicalis (17,28.33%), C. krusei (1,1.66%), C. lusitaniae(1,1.66%), C. ciferrii(2,3.33%). [Figure 1]

Of the 6 blood samples, three samples yielded C. parapsilosis and three yielded C. tropicalis. C. parapsilosis predominated in pus samples (11,45.83%) followed by C. albicans(6,25%), C. tropicalis (4,16.6%),C. ciferri(2,8.3%)and C.lusitaniae (1,4.16%). The major pathogen from urine samples was C. albicans (12,40%) while C. tropicalis accounted for 9(30%), Candida tropicalis for 8(26.6%) and C.krusei for 1(3.33%)case respectively. [Table 1]

All 16 Candida albicans isolates were sensitive to fluconazole (MIC90-0.032-0.19), voriconazole (MIC90 0.04-0.09) and Amphotericin B (MIC90 0.012-0.19). Similar results were obtained for Candida tropicalis.

Only one in 23 Candida parapsilosis isolates showed resistance to fluconazole (MIC90-12).The sensitive MICs varied from 0.047-1.5. All isolates were sensitive to voriconazole (MIC90 0.0023-0.094) and Amphotericin B (MIC90 0.064-1).

The only C.krusei isolate obtained from urine sample was moderately sensitive to fluconazole(MIC90-8) and sensitive to both voriconazole (MIC90-0.25) and Amphotericin B(MIC90-2).

Both C.Cifferri isolates were sensitive to voriconazole(MIC90-0.25-0.5) and Amphotericin B (MIC90 1-1.5) and resistant to fluconazole(MIC90-32)

For both azoles, the essential agreement between VITEK2 system and E test were excellent, ranging from 95% - 100%. For amphotericin B the essential agreement between both tests were 100%

Table 1: Antifungal susceptibilitiy of Candida isolates

Species name	N		Fluconazole			Voriconazole			Amphotericin		
			Sensitive	IM	Resistant	Sensitive	IM	Resistant	Sensitive	IM	Resistant
Candida Albicans	16	Vitek	16(100%)	0(0%)	0(0%)	16(100%)	0(0%)	0(0%)	16(100%)	0(0%)	0(0%)
		Etest	16(100%)	0(0%)	0(0%)	16(100%)	0(0%)	0(0%)	16(100%)	0(0%)	0(0%)
Candida Tropicalis	17	Vitek	17(100%)	0(0%)	0(0%)	17(100%)	0(0%)	0(0%)	17(100%)	0(0%)	0(0%)
		Etest	17(100%)	0(0%)	0(0%)	17(100%)	0(0%)	0(0%)	(100%)	0(0%)	0(0%)
Candida parapsilosis	23	Vitek	(95.5%)	0(0%)	1(4.5%)	22(100%)	0(0%)	0(0%)	22(100%)	0(0%)	0(0%)
		Etest	22(95.6%)	0(0%)	1(4.5%)	22(100%)	0(0%)	0(0%)	22(100%)	0(0%)	0(0%)
Candida Krusei	1	Vitek	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)
		Etest	0(0%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	0(0%)
Candida	1	Vitek	1(100%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Lusitaniae		Etest	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)	0(0%)	1(100%)	0(0%)	0(0%)
Candida Ciferii	2	Vitek	0 (0%)	0(0%)	2(100%)	2(100%)	0(0%))	0(0%)	2(100%)	0(0%)	0(0%)
		Etest	0(0%)	0(0%)	2(100%)	2(100%)	0(0%)	0(0%)	2(100%)	0(0%)	0(0%)

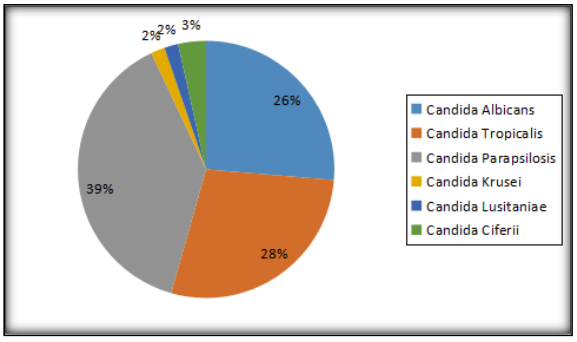

Figure 1 Characterization of isolated Candida spp

DISCUSSION

According to this research, the predominant Candida species isolated were Candida non albicans specifically Candida parapsilosis (38.3%) and Candida tropicalis (28.3%). This is similar to findings by Sachin et al, Taei et al.[13,14]

All the Candida albicans isolates were sensitive to azoles and the non albicans candida spp isolates in our institute showed only low level resistance to fluconazole(15.3%) in contrary to studies published by yang et al,[15] similar findings was observed in south korea by jae et al,[16] Studies have shown that variable degrees of inducible azole resistance have been observed in non albicans candida spp especially candida tropicalis within 90 days of onset of therapy.[17] The samples in our research were collected before the onset of therapy. This might have contributed for the low-level azole resistance in the isolates

When comparing the VITEK 2 system and E Test, similar MIC values were obtained for both methods. The essential agreement between VITEK2 system and E test ranged from 95% to 100% for azoles and 100% for amphotericin B. However the mic values for VITEK system was available by around 14-18 hours with average of 16 hours when compared to 48 hours in E test thus reducing the time for optimizing antifungal treatment decisions. Though both methods produced comparable results, VITEK2 system may be considered as an alternative to E test for antifungal susceptibility testing due to quicker turn around time.

CONCLUSION

Candida non albicans species predominated the isolates. Candida non albicans showed only low level resistance to azoles and all Candida albicans isolates were azole sensitive.The AST-YS01 Vitek 2 card system (bioMérieux) is a reliable standardized automated antifungal susceptibility test and showed comparable results to E test and thus may be used alternately.

REFERENCES

1. Zaoutis, T.E., Argon, J., Chu, J., Berlin, J.A., Walsh, T.J., Feudtner, C. 2005. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. Clin. Infect. Dis., 41: 1232-1239.
2. Tortorano AM, Caspani L, Rigoni Al, Biraghi E, Sicignano A, Viviani MA: Candidosis in intensive care unit: a 20 year survey. J Hosp Infect 2004, 57:8-13.
3. Bhattacharjee P. Epidemiology and antifungal susceptibility of Candida species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016; 2(2): 20-27. DOI: 10.18869/acadpub.cmm.2.2.5
4. Rajgopal S. K. Prevalence and Antifungal Susceptibilities of Candida spp from a South Indian Tertiary Care Hospital. Int J Med Res Rev 2017;5(02):98-104. doi:10.17511/ijmrr. 2017.i02.01.
5. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. J Clin Microbiol. 2010 Apr;48(4):1366-77. doi: 10.1128/JCM.02117-09.
6. J. C. O. Sardi, L. Scorzoni, T. Bernardi. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Medl Microbiology (2013), 62, 10–24
7. Papon N, Courdavault V, Clastre M, Bennett RJ. Emerging and emerged pathogenic Candida species: beyond the Candida albicans paradigm. PLoS Pathog. 2013;9(9):e1003550. doi: 10.1371/journal.ppat.1003550.
8. Deorukhkar SC, Santosh Saini S, Mathew S. 2014. Nonalbicans Candida infection: an emerging threat. Interdisciplinary Perspect. Infect. Dis. 2014: 615958.
9. Ellis D. Amphotericin B: spectrum and resistance. J Antimicrob Chemother. 2002;49(Suppl 1):7–10.
10. Liguori G, Di Onofrio V, Gallé F, Lucariello A, Albano L, Catania MR, et al. Candida albicans identification: comparison among nine phenotypic systems and a multiplex PCR. J Prev Med Hyg. 2010;51(3):121–124.
11. Melhem MS, Bertoletti A, Lucca HR, Silva RB, Meneghin FA, Szeszs MW. Use of the VITEK 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species. Braz J Microbiol. 2014;44(4):1257-66. doi:10.1590/S1517-83822014005000018.
12. Song YB, Suh MS, Ha GY, Kim H. Antifungal Susceptibility Testing with Etest for Candida Species Isolated from Patients with Oral Candidiasis. Ann Dermatol. 2015;27(6):715–720.
13. Sachin C. Deorukhkar, Santosh Saini, Stephen Mathew, "Non-albicans Candida Infection: An Emerging Threat", Interdisciplinary Perspectives on Infectious Diseases, vol. 2014, Article ID 615958, 7 pages, 2014. https://doi.org/10.1155/2014/615958)
14. Taei M., Chadeganipour, M. & Mohammadi, R. An alarming rise of non-albicans Candida species and uncommon yeasts in the clinical samples; a combination of

ISSN (O): 2687-5365; ISSN (P): 2753-6556

various molecular techniques for identification of etiologic agents. BMC Res Notes **12,** 779 (2019). https://doi.org/10.1186/s13104-019-4811-1)

15. Yang Y., Ho, Y., Cheng, H., Ho, M., & Lo, H. (2004). Susceptibilities of Candida Species to Amphotericin B and Fluconazole: The Emergence of Fluconazole Resistance in Candida tropicalis. Infection Control & Hospital Epidemiology, 25(1), 60-64. doi:10.1086/502294.
16. Jae Il Yoo, Chi Won Choi, Kyeong Min Lee, Young Kwon Kim, Tae Un Kim, Eui-Chong Kim, Sae Ik Joo, Seon Han Yun, Yeong Seon Lee, Bong Su Kim, National surveillance of antifungal susceptibility of *Candida* species in South Korean hospitals, *Medical Mycology*, Volume 47, Issue 5, August 2009, Pages 554–558, https://doi.org/10.1080/13693780802354037).
17. Saikat Paul, Shreya Singh, Dipti Sharma, Arunaloke Chakrabarti, Shivaprakash M. Rudramurthy, Anup K. Ghosh,Dynamics of in vitro development of azole resistance in Candida tropicalis,Journal of Global Antimicrobial Resistance,Volume 22,2020,Pages 553-561,ISSN 2213-7165, https://doi.org/10.1016/j.jgar.2020.04.018. (https://www.sciencedirect.com/science/article/pii/S2213716520301107)

ISSN (O): 2687-5365; ISSN (P): 2753-6556