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Rapid Identification of Candida Species Isolated from Various Clinical Samples of Diabetic Pregnant Women and Their Susceptibility Pattern by Using VITEK 2 in a Tertiary Care Hospital, Jodhpur, Rajasthan

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Abstract

Introduction: *Candida albicans* is most common fungal infection in gestational diabetic women. Their rapid identification and proper treatment is necessary. In this study, we evaluated the performance of VITEK 2 for rapid identification of *Candida albicans* and *non albicans Candida* and antifungal sensitivity pattern. This cross sectional analytical study was performed on 75 diagnosed diabetic pregnant women who were admitted to Umaid hospital, Jodhpur city, Rajasthan from November 2012 to July 2013.

Method: All samples were identified to species level by VITEK 2(Automated Method). A total of 225 samples were taken from diabetic pregnant women from three different sites (urine, vaginal swab & throat swab).

Result: In 125 (55.55%) samples growth of Candida species were observed. *C. famata* (29.2%) was the most common species identified by VITEK followed by *Stephanoascus ciferrii* (27.2%) and *Candida albicans* (12.8%). *C. albicans, C. tropicalis* and *c. parapsilosis* showed susceptibility to all antifungal drugs whereas *C. glabrata* showed decrease sensitivity to VR, FU, CSP and AP but sensitive to flucytosine. *C. famata* did not response to any antifungal drug. *Stephanoascus ciferrii* showed decreased sensitivity to VR and FU.

Conclusion: Thus, *non albicans candida* infection is increasing in gestational diabetic pregnant women. Therefore, accurate and rapid identification and susceptibility pattern is necessary for management of infection. VITEK system is certainly the more efficient method over the conventional method as the results are available in a shorter period of time as compare to conventional methods and provide susceptibility profile & MIC at the same time.

Keywords: *Candida albicans*, VITEK 2, *C. famata, Stephanoascus ciferrii*

Introduction

Candida is an asexual, diploid, dimorphic fungus that is present on human body. *Candida* species are part of the normal flora of skin, gut and genitals and is capable of causing a variety of infections. These

organisms cause superficial and deep-seated mycoses such as cutaneous, mucocutaneous, subcutaneous, or systemic candidiasis. General risk factors for *Candida* infections are associated with compromised immune system, diabetes mellitus, and iatrogenic factors like antibiotic use, indwelling devices, intravenous drug use.[1] Diabetes mellitus is the leading endocrine dysfunction which favours *Candidial* infection. Diabetes and *Candida* infections often occur during pregnancy.

Till the present time, *Candida albicans* was considered as the most frequently isolated *Candida* species but non-albicans Candida have now become predominant and become pathogenic for human being. [2,3,4]

Rapid identification of yeasts provides timely information for proper treatment of patient. Identification of yeasts is mainly based on biochemical reaction conventially. The Vitek 2 system (bioMerieux, Marcy Etoile, France) first introduced a fluorometric and then a colorimetric card for the rapid identification of yeast species. The performance of both cards has been evaluated in several studies [5,6,7,8,9,10] and was shown to be useful for species identification. The Vitek 2 manufacturer recommends different fungal media for isolate pure colony of *Candida*, such as Sabouraud dextrose agar, Trypticase soy agar, and yeast selective agar. Currently, the most common agar media used for routine diagnostics, chromogenic media and Sabouraud agar with antibiotics, Only one study has comparatively evaluated the performance of the new colorimetric Vitek 2 YST card using two different source media for a limited number of isolates.[5]

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The VITEK 2 System is an integrated modular system that consists of a filling-sealer unit, a reader incubator, a computer control module, a data terminal, and a multicopy printer. The system detects organism growth and metabolic changes in the micro wells of thin plastic cards by using fluorescence–based technology (a colorimetric-based instrument was introduced in late 2004).[11]

In this study, we evaluated the performance of Vitek 2 automated system for the identification and antifungal sensitivity pattern of medically important yeasts in a routine clinical microbiology laboratory.

Materials and methods

This cross sectional study was conducted through November 2012 to July 2013 at department of Microbiology, Dr. S.N. Medical College, and associated Group of Hospital, Jodhpur Rajasthan. Patient's data were obtained from hospital record. The following data were recorded: age, diabetic status, type of samples and age of gestation.75 diagnosed diabetic pregnant women who were attending to antenatal clinic or admitted to antenatal ward to Umaid hospital Jodhpur, were taken for the study. Diabetic affliction criterion was random blood sugar level higher than 110 mg/dl. A total of 225 samples of urine, vaginal swab and throat swab (75 from each site) were collected from diabetic pregnant women.

All samples were subjected to fungal culture media SDA with antibiotic (sabouraud's dextrose agar with chloramphenicol) and incubated at 37ºc. When pure colonies grown on the SDA, They were subjected to Gram stain and the yeasts were further identified. Pure growth of isolated colony was subjected to species identification by VITEK 2. All the isolates were identified to the species level by Vitek 2 YST identification card (bioMerieux, France). Antifungal sensitivity was also performed against Amphotericin B (AP), Flucytosine (FLC), Voriconazole (VRC), Fluconazole (FU), Caspofungin (CSP) using ASTYS06 (bioMerieux, France). Results were

interpreted as per CLSI. (Clinical Laboratory Standards Institute)

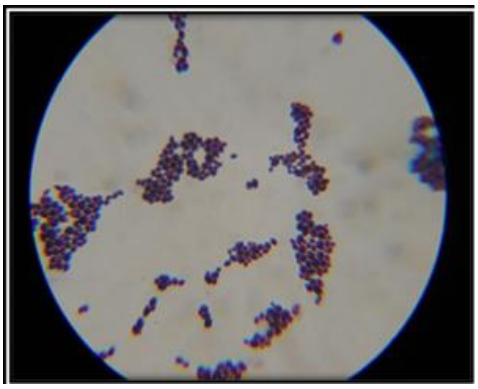

Figure 1: Gram staining showing budding yeast cell.

Figure 2: The automated - Vitek -2 system.

VITEK 2-: The VITEK ID-YST card consists of 64 wells with 47 fluorescent biochemical tests. They comprise 20 carbohydrate assimilation tests : adonitol(ribitol), D-trehalose, D-cellobiose,dulcitol, D-galactose, D-glucose, lactose, D-maltose, D-mannitol, D-melibiose, D-melezitose, palatinose, D-raffinose, L-rhamnose, sucrose, salicine, L-sorbose, D-sorbitol, D-L-lactate, and succinate.The six organic acid assimilation tests are N-acetyl-glucosamine, methyl-a-D-glucopyranoside, citrate, D-galacturonate, D-gluconate, and mono-methyl ester succinate. The eight substrates for the detection of the oxidases are coupled with 4-methylumbelliferone (4MU): a-galactoside–4MU, a-glucoside–4MU, a-mannoside–4MU, b-galactoside–4MU, b-glucoside–4MU, b-glucuronide– 4MU, b-N-acetyl-glucosaminide–4MU, and b-xyloside–4MU. The nine substrates for the detection of arylamidases are coupled with 7-amino-methylcoumarin (7AMC): glycine-7AMC, hydroxyproline-7AMC, H-lysine-alanine–7AMC, g-glutamyl-transferase–7AMC, H-glycine-glycine–7AMC, histidine-7AMC, isoleucine-7AMC, proline-7AMC, and valine-7AMC. The four miscellaneous tests are phosphatase, urea, nitrate, and actidione.[24]

A 2.0 McFarland standard suspension of *Candida* strain was prepared, YST ID card used for species identification and yeast susceptibility testing (YST) card was used for AST i.e., AST-YS06 kits. This card or cassette consists of following antifungal powders (5-flucytosine, fluconazole, voriconazole, amphotericin B and caspofungin) in small reaction tubes. Minimum inhibitory concentration (MIC) of 5-flucytosine, fluconazole, voriconazole, amphotericin B and caspofungin was determined by VITEK-2 Compact (Biomerieux, France).The results provided by VITEK-2 were interpreted as sensitive, intermediate or resistant for that particular antifungal drug.[24]

Results

A total of 125 *Candida* isolates were identified form urine, vaginal swab and throat swab by VITEK 2 which belong to 9 *Candida* species. The majority of the isolates were from vaginal swab 56 (44.8%) followed by urine 46 (36.8%) and throat swab 23 (18.4%). (Table -1)

Table 1: Distribution of *Candida* isolates in clinical samples.

Sample	No. of *Candida* isolates
Urine	46/125 (36.8%)
Vaginal Swab	56/125 (44.8%)
Throat Swab	23/125 (18.4%)

Table 2: Distribution of *Candida* species identified by VITEK 2 YST cards.

Isolate species	Total species		Urine		vaginal swab		throat swab	
	No.	%	**No.**	%	**No.**	**%**	**No.**	**%**
C. albicans	16	12.8	7	15.2	7	12.5	2	8.70
C .krusei	5	4.0	1	2.17	3	5.3	1	4.35
C. tropicalis	2	1.6	0	0	1	1.78	1	4.35
C. glabrata	6	4.8	3	6.52	3	5.36	0	0
C. famata	37	29.6	11	23.9	18	32.15	8	34.7
C. parapsilosis	2	1.6	1	2.17	0	0	1	4.35
Stephanoascus ciferrii	34	27.2	16	34.7	13	23.21	5	21.74
C. laurentii	1	0.8	0	0	1	1.78	0	0
Unidentified	22	17.6	7	15.2	10	17.86	5	21.7
Total	125	100	46	100	56	100	23	100

The most common species among 125 isolates were *Candida famata* (29.6%) followed by *Stephanoascus ciferrii* (27.2%) and *Candida albicans* (12.8%). (Table 2)

Table 3 shows the sensitivity pattern of isolated *Candida* species to various antifungals. *C. albicans* showed 100% sensitive to caspofungin, 75% to fluconazole, 68.7% for voriconazole and 62.5% to amphotericin B & flucytosine. *C. tropicalis* showed 100% sensitivity to caspofungin and amphotericin B, 50% sensitivity to flucytosine and voriconazole. *C. krusei* showed 100% sensitivity to caspofungin, 80% sensitivity to amphotericin B & flucytosine and 60% sensitivity to voriconazole. *Stephanoascus ciferrii* were 97% resistant to fluconazole, 91% resistant to voriconazole and 70% sensitive to caspofungin.

Discussion

The present study was conducted in the Department of Microbiology Dr.S.N.Medical College & Associate group of Hospitals, Jodhpur, and Rajasthan. Out of 225 samples, in 125 (55.55%) samples growth of *Candida* species were observed and in the rest of the samples were no growth. *Candida* infection is higher in diabetic pregnant women because increase level of glucose in diabetes during pregnancy favours the growth of *Candida* species which probably showed increase prevalence of *Candida* infection in diabetic pregnant women. It is in accordance to the study conducted by Mirela babić et al (2010).[12] they found that pregnant women were more prone to *Candidal* infection. During pregnancy, vagina is more sensitive, and the infections occur significantly more often. The high incidence of vaginitis in pregnant women is related to levels of estrogens, which is in turn considered the primary factor for the occurrence of infection.

In this study, the most common sample from which *Candida* species were isolated was vaginal swab. Similar study was done by Reza faraji et al (2012)[13] they found that Vulvovaginal candidiasis was more prevalent in women with diabetes. Increased glucose levels in genital tissues enhance yeast adhesion and growth. Vaginal epithelial cells bind to Candida with greater propensity in diabetic pregnant women.

Several studies have shown a considerable increase in non-*albicans Candida* infection. A study by Saldhana et al. [14] showed that non-*albicans Candida* were isolated more frequently (53%) than *C. albicans* (47%).

Table 3: Sensitivity Pattern of Candida isolates by VITEK 2.

Isolate species	Total no. of spp.	AP			FLC			VR			FU			CSP		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. albicans	16	10 (62.5%)	6	-	10 (62.5%)	6	-	11 (68.7%)	5	-	2 (75%)	-	4	16 (100%)	-	-
C .krusei	5	4 (80%)	1	-	4 (80%)	1	-	3 (60%)	1	1	1 (20%)	-	4	5 (100%)	-	-
C. tropicalis	2	2 (100%	-	-	1 (50%)	-	1	1 (50%)	-	1	-	-	2	2 (100%)	-	-
C. glabrata	6	-	6	-	3 (50%)	3	-	-	4	2	-	4	2	-	4	2
C. parapsilosis	2	2 (100%)	-	-	2 (100%)	-	-	2 (100%)	-	-	2 (100%)	-	-	2 (100%)	-	-
Stephanoascus ciferrii	34	19 (55.8%)	15	-	-	-	-	3 (8.82%)	-	31	19 (2.9%)	-	33	24 (70.5%)	-	10
C. laurentii	1	1 (100%)	-	-	1 (100%)	-	-	1 (100%)	-	-	1 (100%)	-	-	1 (100%)	-	-

AP- Amphotericin B, FLC-Flucytosine, VRC-Voriconazole, FU- Fluconazole, CSP-Caspofungin
(S-sensitive, I-Intermediate, R-resistant)

These results were in agreement with the findings of the study by Mokaddas et al.[15] who also showed the non-*albicans Candida* incidence (60.5%) was higher than that of *C. albicans* (39.5%).These findings suggest that non-*albicans Candida* species are emerging as important pathogens. In our study, we found *C. famata* (29.6%) was the most common species followed by *Stephanoascus ciferrii* (27.2%) and *C. albicans* (12.8%).

In this study, *Stephanoascus ciferrii* (34.7%) were highest in urine sample followed by *C. famata* (23.9%) and *C. albicans* (15.2%). In vaginal swab, (32.15%) *C. famata*, (23.2%) *Stephanoascus ciferrii* and (12.5%) *C. albicans* were identified by Vitek. In throat swab Vitek identified (34.7%) *C. famata*, (21.7%) *Stephanoascus ciferrii* and (8.70%) *C.albicans*. A Study conducted by Sankarankutty J et al.[16] found non-*albicans candida* was highest in urine (48% *c. tropicalis*).

C. albicans, C. tropicalis and *c. parapsilosis* showed susceptibility to all antifungal drugs whereas *C. glabrata* had decreased sensitivity to VR, FU, CSP and AP but sensitive to flucytosine. According to a study done by Sowmya G. S et al.[17] Concluded that all candida isolates were sensitive to voriconazole, amphotericin B & caspofungin. *Stephanoascus ciferrii* showed decreased sensitivity to VR and FU. A case report by Tayfur D.[18] found that *Stephanoascus ciferrii* cause cutaneous and systemic infection in newborn and was resistant to AP and FU.

VITEK 2 also provides MIC for antifungal. *C. albicans* had an MIC of Flucytosine <=1 (sensitive), Voriconazole MIC <=0.12 (sensitive), Amphotericin B MIC was 0.5 (sensitive) & >=16 (resistance), Fluconazole MIC was 2 (sensitive) & >=64 (resistance) and Caspofungin MIC was <=0.25 (sensitive). *C. krusei* had an MIC of Flucytosine <=1 (sensitive), Voriconazole MIC <=0.12 (sensitive), Amphotericin B MIC 0.5 (sensitive) & >=16 (resistance), Fluconazole MIC 2 (sensitive) & >=64 (resistance) and Caspofungin MIC <=0.25 (sensitive). *C. glabrata* had an MIC of Flucytosine <=1 (sensitive), Voriconazole MIC <=0.12 (sensitive) & >=0.8 (resistance), Amphotericin B MIC 0.5 (sensitive) & 8 (resistance), Fluconazole MIC <=1 (sensitive) & >=64 (resistance) and Caspofungin MIC <=0.25 (sensitive). *C. parapsilosis* had an MIC of Flucytosine <=1 (sensitive), Voriconazole MIC <=0.12 (sensitive), Amphotericin B MIC 0.5 (sensitive), Fluconazole MIC <=1 (sensitive) and Caspofungin MIC <=0.25 (sensitive). *C. tropicalis* had an MIC of Flucytosine <=1 (sensitive), Voriconazole MIC <=0.12 (sensitive), Amphotericin B MIC 0.5 (sensitive), Fluconazole MIC 1 (sensitive) and Caspofungin MIC <=0.25 (sensitive). *Stephanoascus ciferrii* had an Amphotericin B MIC 0.5 (sensitive), 2 intermediate and 8 (resistance) and Caspofungin MIC <=0.25 (sensitive).

The new species *C. famata* (synonym: Debaryomyces hansenii) associated with eye[19] and intravenous catheter infections[19], fungaemia[20,21] and peritonitis[22] respectively. Nicolas p. et al.[23] found *C. famata* shows moderate resistant to polyenes and azoles in contrast to this study which found *C. famata* do not respond to any antifungal.

In conclusion, in the present study, the prevalence of non-*albicans Candida* infection was more as compared to *C.albicans* and among the non-*albicans Candida*, *C. famata* was most common species. Conventional identification methods are still considered to be the reference standard for the identification of yeast isolates, but are laborious and time-consuming, and are suited better to research than to clinical laboratories. The Vitek 2 system identifies most clinically important *Candida* spp. within 15 h, and appears to be an excellent alternative identification

method for clinical laboratories performing fungal diagnostics.

References

1. Mohandas V. Ballal M. Distribution of Candida species in different clinical samples and their virulence: Biofilm formation, proteinase and phospholipase production: A study on hospitalized patients in Southern India. *J Glob Infect Dis*. 2011; 3(1): 48.
2. Adhikary R. Joshi S. Species distribution and antifungal susceptibility of Candidaemia at multi super- speciality center in southern India. *IJMM* 2011;29:309-11.
3. Chander J. A textbook of medical mycology (Mehta publishers, Nariana II, New Delhi, India). 3rd edition 2009; 26683.
4. Topley WWC, Wilson CS. Candidiasis, in Topley Wilson's Medical mycology. Chapter 30:579- 13.
5. Aubertine, C. L. M. Rivera. S. M. Rohan, and D. H. Larone. Comparative study of the new colorimetric VITEK 2 yeast identification card versus the older fluorometric card and of CHROMagar Candida as a source medium with the new card. *J. Clin. Microbiol*. 2006; 44:227–228.
6. Ca´rdenes-Perera, C. D., A. Torres-Lana, R. Alonso-Vargas, M. D. Moragues-Tosantas, J. Ponton-San Emeterio, G. Quindos-Andres, and M. P. Arevalo-Morales. Evaluation of API ID 32C and VITEK-2 to identify Candida dubliniensis. Diagn. *Microbiol. Infect. Dis*. 2004; 50:219–221.
7. Graf, B., T. Adam, E. Zill, and U. B. Gobel. Evaluation of the VITEK 2 system for rapid identification of yeasts and yeast-like organisms. *J. Clin. Microbiol.* 2000;38:1782–1785.
8. Loı¨ez, C. Wallet F. Sendid B. and Courcol JR. Evaluation of VITEK 2 colorimetric cards versus fluorimetric cards for identification of yeasts. Diagn. *Microbiol. Infect. Dis*. 2006; 56:455–457.
9. Meurman, O. Koskensalo A. and Rantakokko-Jalava K. Evaluation of Vitek 2 for identification of yeasts in the clinical laboratory. *Clin. Microbiol. Infect*. 2006; 12:591–593.
10. Sanguinetti, M. Porta R. Sali M. Sorda M. Pecorini G. Fadda G. and .Posteraro B.2007.Evaluation of VITEK 2 and RapID yeast plus systems for yeast species identification: experience at a large clinical microbiology laboratory. *J. Clin. Microbiol*. 45:1343–1346.
11. Konemen E, Allen S, Procop G, Woods G, Janda W, Sixth Edition Colour Atlas and Textbook of diagnostic microbiology: The Enterobacteriaceae-Semi automated and Automated Identification System, ch. 6, p:292-293.
12. Mirela B. Mirsada H. candida albicans and non-albicans species as etiological agent of vaginitis in pregnant and non-pregnant women, *bosnian journal of basic medical sciences.* 2010; 89-97.
13. Reza F. Mehr Ali R. Fatemeh R. and Masoud H. Prevalence of vaginal candidiasis infection in diabetic women Diabetes, *African Journal of Microbiology Research,* 2012; Vol. 611, pp. 2773-2778.
14. Dharwad S, RM SD. Species identification of Candida isolates in various clinical specimens with their antifungal susceptibility patterns. *J Clin Diagn Res*. 2011; (suppl-1); 5(6): 1177-81.
15. Mokaddas EM, Al-Sweih NA, Khan ZU. Species distribution and antifungal susceptibility of Candida bloodstream isolates in Kuwait: a 10-year study. *J Med Microbiol*. 2007; 56(2): 255-9
16. Sankarankutty J. Vipparti H. Candida Species Isolated from Various Clinical Samples and Their Susceptibility Patterns to Antifungals. *J Med Microbiol Infec Dis* 2013, 1(1): 22-26.

17. Sowmya G. S, Madhuri Kulkarni, Sumana M. Speciation and antifungal susceptibility pattern of candida isolates in a tertiary care hospital. *International Journal of Advances in Science and Technology (IJAST),* 2014; 2(3).
18. Tayfur D. Mehmet K. Ahmet O. Mustafa A. The first case of Stephanoascus ciferrii in new born. *Nobel Medicus* December 2015.
19. Hazen KC. New and emerging yeast pathogens. *Clin Microbiol Rev* 1995; 8: 462-478.
20. Krcmery V, Kunova A. Candida famata fungemia in a cancer patient: case report. *J Chemother* 2000; 12: 189-190.
21. Carrega G, Riccio G, Santoriello L, Pasqualini M, Pellicci R. Candida famata fungemia in a surgical patient
25.
successfully treated with fluconazole. *Eur J. Clin. Microbiol Infect Dis*. 1997; 16: 698-699.
22. Quindos G, Cabrera F, Arilla MC, et al. Fatal Candida famata peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis who was treated with fluconazole. *Clin Infect Dis* 1994; 18: 658-660.
23. Nicolas P. Vincent C. Marc C. Benette RJ, et al. Emerging and Emerged Pathogenic Candida Species: Beyond the Candida albicans Paradigm, PLoS Pathog 9(9): e1003550.
24. Barbara G. Thomas A. Edith Z. Ulf bG. et al, Evaluation of the VITEK 2 System for Rapid Identi®cation of Yeasts and Yeast-Like Organisms . *J. of Clin. Microbiol,* 2000; 38 (5): 1782-1785.