Int.J.Curr.Microbiol.App.Sci (2016) *5*(3): 579-589

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 3(2016) pp. 579-589**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.503.067

A Comparative Analysis on Identification and Antifungal Susceptibility Profile of Candida Isolates Using Conventional and Automated Techniques

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Keywords

Candida species, Antifungal susceptibility testing, VITEK-2, E-test.

Article Info

Accepted:
18 February 2016
Available Online:
10, March 2016

A B S T R A C T

Candida species are the most common fungal pathogen isolated from clinical samples. Of late, improper use of antifungal drugs in critically ill patients has favoured antifungal drug resistance amongst Candida. To isolate and identify *Candida* species from hospitalized patients and determine their antifungal susceptibility profile by conventional (Epsilometer i.e., E-test) and automated (VITEK-2) methods. This cross sectional study was carried out from December 2012 to June 2014 from hospitalized patients at a tertiary care centre, in which 50 non-repetitive clinical isolates of Candida were analysed. The identification was carried by conventional methods as per standard laboratory protocols and by VITEK-2 Compact (Biomerieux, France). The antifungal susceptibility testing (AFST) of these isolates was carried out by E-test and VITEK-2 Compact (YST cards- AST YS01). Out of 50 isolates, 36 (72%) were non-albicans Candida and 14 (28%) were *C.albicans*. AFST by VITEK-2 showed that only 2 isolates (4%) were resistant to fluconazole and E-test showed that 24 isolates (48%) were resistant to fluconazole and voriconazole but all were sensitive to amphotericin B. There was substantial agreement of 80% (95% CI of 69 – 91%) for identification and less than chance agreement for AFST between VITEK-2 and E-test. We conclude that E-test can be a simple and convenient method for AFST when compared to VITEK-2. We cannot rely only on VITEK-2 for AFST. Identification and AFST of Candida isolates must be carried out for optimum patient care.

Introduction

Candida speciesare part of the normal flora of skin, gut and genitals and is capable of causing a variety of infections and is emerging as an important nosocomial pathogen. They are the most common cause of fungal infections leading to invasive life

threatening diseases. At present, candidal infections are the fourth leading cause of blood stream infections and one of the common opportunistic pathogen (1,2).The increasing incidence of HIV infections, widespread use of antibiotics, organ transplantation, use of immunosuppressive agents and emergence of resistance to antifungal agents has contributed to the increased incidence of Candida infections.

The increasing incidence of invasive candidiasis in critically ill patients in ICU, acute wards, oncology wards and resistance to antifungal drugs is becoming worrisome.Long term use of azoles in the prophylaxis of systemic fungal infections in bone marrow transplant patients and for long-term suppressive therapy in patients with AIDS is an important factor in the selection of isolates that exhibit increased resistance to azole therapy (3,4).

Till recent times,*Candida albicans* was considered as the most frequently isolated Candida species but non-albicans Candida have now become predominant (5,6,7).The commonly isolated non-albicans Candidaare *C. tropicalis*, *C.parapsilosis*, *C. guilliermondii*, *C. dubliniensis*, *C. glabrata*, *C.lusitaniae*, *C.kefyr* and *C.krusei*. Some of the non-albicans Candida are intrinsically resistant to fluconazole (*C.krusei*) and some of them show resistance through enzyme modification to fluconazole (*C.glabrata*) (6,7,8,9,10). In view of this, reporting the infecting species of Candida alongwith antifungal susceptibility testing is of utmost importance. We studied the prevalence of various Candida species in hospitalized patients at a tertiary care centrealong with their antifungal susceptibility pattern to commonly used antifungal drugs by two methods namely, E-test, a conventional method and secondly by VITEK-2, an automated system (5,9,10).

Materials and Methods

Sampling and Identification

A cross sectional study was carried out from Dec 2012 to Jun 2014, in which a total of 50 non-repetitive clinical isolates of Candida were included from various clinical samples like blood, urine, muco-cutaneous samples (nail clippings, oral scrapings and high vaginal swabs), pleural fluids and continuous ambulatory peritoneal dialysis (CAPD) fluids. The blood samples were collected in BACTEC (BD BACTEC Mycosis IC/F, Ireland) and brain heart infusion (BHI) bottlesunder aseptic precautions. Clean catch midstream urine, urine from catheter, body fluids and CAPD dialysate were collected in wide mouthed sterile universal containers. Samples were transported and processed immediately as per standard laboratory protocols.

The organism was identified using conventional methods that included Gram stain,colony morphology in Sabouraud dextrose agar (SDA) with and without chloramphenicol. Germ tube test was used to differentiate *C.albicans* from non-albicans Candida. CHROM agar (HI media) inoculation was done for differentiating the *Candida* species exhibiting differently coloured colonies, example: *C.albicans*- light green colour, *C.tropicalis*- dark blue colour, *C.krusei*- rough pink colour, *C.glabrata*- smooth pink colour, *C.parapsilosis*- creamy white to pale pink, *C.dubliniensis*- dark green(Fig 1). The sugar fermentation test was carried out using 2% sugars (Glucose, sucrose, maltose, trehalose and lactose). The sugar assimilation test was carried out with eight different sugar discs of 2% concentration (Glucose, sucrose, maltose, lactose, trehalose, xylose, cellobiose and dulcitol) in yeast nitrogen base agar (HI media) which is a

carbohydrate deficient medium and the test candida strains were inoculated by pour plate method (Fig 2). Cornmeal agar with Tween-80 inoculation (HI media) were done in the Dalmau plates which is a nutrient deficient medium, used for observing sporulation and formation of blastoconidia, pseudohyphae and chlamydospore under 10x and 40x magnification (Fig 3). These conventional methods are the gold standard for identification of the candida in this study. ATCC Candida strains were procured from PGI Chandigarh and used as controls- *Candida parapsilosis* (ATCC 22019); *Candida krusei* (ATCC 6258); *Candida albicans* (ATCC 90028)

Identification of yeast and yeast like organisms was simultaneously carried out by automated system [VITEK-2 Compact (Biomerieux, France)] using VITEK-2 cards (ID-YST cards)in parallel. The ID-YST cards used for yeast identification consist of two cassettes or cards. The first card is for identification (ID card) that consists of various sugars for assimilation and biochemical reactions in small reaction tubes. The final identification was done by matching with the yeast data library in the VITEK-2 systems software in the form of binary digit representation like 1 (positive, +) or 0 (negative, -). The second card is for yeast susceptibility testing (YST card).

Antifungal Susceptibility Testing (AFST)

Antifungal susceptibility testing (AFST) was carried out by conventional method (E-test) and automated method (VITEK-2).

E-test:A 0.5 McFarland standard suspension of Candida strain was prepared and E-strips (AB Biodisk, Solna, Sweden) of amphotericin B, voriconazole and fluconazole were applied over the RPMI 1640 agar with L-glutamine but without sodium bicarbonate (HI media) at pH 7.0. MIC of fluconazole, voriconazole and amphotericin B was determined.The zone edge intersecting the graded strip at the minimum concentration of the antibiotic was interpreted as MIC (Fig 4 & 5). All the results were interpreted according to CLSI M27-S4 (2012)(17).

VITEK-2:A 2.0 McFarland standard suspension of Candida strain was prepared and yeast susceptibility testing (YST) card was used for AFST i.e., AST-YS01 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, depending upon the yeast identified and by correlating the MIC values with CLSI and EUCAST guideline installed in VITEK-2systems software. But VITEK-2 does not give the result as per the break point levels for each antifungal drug and the exact MIC value is not determined.

Statistical Analysis

The concordance between conventional and automated methods regarding identification and antifungal susceptibility testing (AFST) of Candida isolates was measured by kappa co-efficient using online software available at http://graphpad.com/ quickcalcs/ kappa1.cfm. as follows:(22)

(a) Less than chance agreement: <0

(b) Slight agreement: 0.01-0.20 (1-20%)

(c) Fair agreement: 0.21-0.40 (21-40%)

(d) Moderate agreement: 0.41-0.60 (41-60%)

(e) Substantial agreement: 0.61-0.80 (61-80%)

(f) Almost perfect agreement: 0.81-0.99 (81-99%)

Results and Discussion

In this study, the most common sample from which *Candida* species were isolated was urine (24) followed by blood (13), muco-cutaneous specimens (08) and other body fluids (05) such as pleural and CAPD fluids (Fig 6). The 36 out of 50 isolates were non-albicans Candida (*C.tropicalis*- 21, *C.parapsilosis*- 11, *C.glabrata* - 02, *C.guilliermondii*- 01, *C.kefyr*- 01) and the rest 14 were *C. albicans*(Fig 7).All isolates exhibited gram positive budding yeast forms with pseudohyphae except *C.glabrata* (budding yeast forms only). The germ tube test effectively differentiated *C.albicans* from non-albicans Candida. The CHROM agar inoculation showed different coloured colonies depending on the species. The sugar fermentation and assimilation reactions and cornmeal agar with Tween-80 inoculation differentiates between different candida species.

Comparison between conventional method and automated method (VITEK-2) in identification was done. Out of 50 isolates, 21 were identified as *C.tropicalis* by conventional method whereas 22 as *C.tropicalis* by VITEK-2. A total of 14 isolates were identified as *C.albicans* by conventional method whereas VITEK-2 identified only 09 as *C.albicans*. Conventional methods identified 11 isolates as *C.parapsilosis* whereas VITEK-2 identified 09 as *C.parapsilosis*. Two *C.parapsilosis* isolate were misidentified as *C.lusitaniae* and *C.tropicalis* by VITEK-2. Similarly *C.albicans*, *C. glabrata* and *C.guilliermondii* were misidentified as *C. famata* by VITEK-2 Compact. So between two methods, 10 out of 50 isolates showed discrepancy in identification (Table 1).

AFST was done for 50 isolates by both VITEK-2 and E-test method, out of which VITEK-2 identified only 02 isolates as resistant to fluconazole (*C.guilliermondii* and *C.lusitaniae*), whereas, 24 isolates were found to be resistant to fluconazole and voriconazole and all isolates were sensitive to amphotericin B (Fig 6 & 7) by E-test method. Out of these 24 resistant isolates, 14 were *C.tropicalis*, 05 were *C.albicans*, 04 were *C.parapsilosis* and 01 was *C.glabrata* (Table 2). The results of minimum inhibitory concentration (MIC) values were interpreted as per CLSI M27-S4 (2012) (16).

The Statistical Analysis

Statistical analysis was carried out for conventional and automated methods, which showed "Substantial agreement" of 80% (95% CI of 69 – 91%) in identification of 40 out of 50 isolates. Ten discrepant isolates were therefore excluded for calculation of agreement and confidence interval (CI) for antifungal susceptibility testing. Two isolates which showed fluconazole resistance by VITEK-2 were sensitive by E-test. Twenty four isolates (48%) were found to be resistant to fluconazole and voriconazole by E-test, whereasVITEK-2identified all of them as sensitive. Therefore, there was no agreement ("less than chance agreement") between VITEK-2 and E-test for antifungal susceptibility testing (22).

Candida species, previously considered a harmless colonizer, have now emerged as

significant pathogens. They are considered as the most common opportunistic fungus. *Candida* species are the most common cause of fungal infections leading to invasive life threatening diseases and it is the fourth leading cause of blood stream infections (2,5,6).Candida colonizes the GI tract of more than 90% of healthy humans. *C.albicans* earlier accounted for majority of isolates in clinical samples, but in recent years non-albicans Candida have become more common, probably because of their greater antifungal drug resistance (6,7,8).Isolates of *C.albicans* were predominantly sensitive to amphotericin B when compared to non-albicans Candida despite the use of polyene antifungal drugs for over five decades. The most common nosocomial infection caused by Candida is urinary tract infection (associated with instrumentation and catheterization) followed by blood stream infections, oral thrush and skin infections.

The increasing incidence of HIV infections, wide spread use of antibiotics, organ transplantation and use of immunosuppressive agents continue to be important factors responsible for the increasing incidence of Candida infections. Furthermore, Candida has assumed greater clinical importance because of their increasing resistance to various antifungal agents (3,4,5,6,7).Some of the non-albicans Candida are intrinsically resistant to fluconazole (*C.krusei*) and some are resistant to fluconazole due to enzyme modification (*C.glabrata*). Therefore, reporting the infecting species of Candida along with antifungal susceptibility pattern must be carried out for optimum patient care and outcome(6,7,8,9,10).

This study had a sample size of 50 Candida isolates with a distribution of 24 (48%) from urine, 13 (26%) from blood, 08 (16%) from muco-cutaneous specimens and 05 (10%) from other body fluids (Pleural and CAPD fluids). A study carried out on 102 patients with candidiasis at New Delhi also obtained similar sample distribution with predominant sample being urine followed by blood and oral scrapings (11).

Out of 50 isolates, 36 (72%) were non albicans Candida and 14 (28%) were *C.albicans*. So the prevalence of non-albicans Candida was more when compared to *C.albicans*. This observation is in consonance with the changing trend seen both in India and across the world. Rani *et al*(2002), Chakrabarti A *et al*(2002), Agarwal *et al* (2004), Capoor *et al*(2005)and Krcmery V *et al* (2002) also showed similar shift from *C.albicans* to non-albicans Candida whereas Wingard *et al* (1995) found *Candida albicans* as the predominant isolates (6,7,8,9,10,11,12,13).

In this study, among the non-albicans Candida, *C.tropicalis* (42%) was the most commonly isolated species followed by *C.parapsilosis* (22%) and others (8%) which includes *C.glabrata*(4%), *C.guilliermondii* (2%) and *C.kefyr*(2%).

Other studies carried out in India also reported *C.tropicalis* to be the predominant non-albicans Candida (8,9,10,11,12). In this study, 06 out of 50 isolates (*C.glabrata*- 02, *C.albicans*- 02, *C.kefyr*- 01, *C.guilliermondii*- 01) were misidentified as *C.famata* by VITEK-2. Studies carried out by Jensen *et al* (2011) and Castanheira *et al* (2013)also reported similar misidentification of *C.albicans*, *C.glabrata* and *C.guilliermondii* as *C.famata*by VITEK-2(14,15).The correct identification of *Candida* species assumes importance not only for epidemiological purposes but also for management due to variation in antifungal susceptibility.

Table.1 Discordance in Identification between Conventional and Automated Method (VITEK-2)

Candidaspecies	Conventional methods	Automated method (VITEK-2)
Candida tropicalis	21	22
Candida albicans	14	09
Candida parapsilosis	11	09
Candida famata	00	06
Candida guilliermondii	01	03
Candida glabrata	02	00
Candida kefyr	01	00
Candida lusitaniae	00	01

Table.2 Discordance in Anti-Fungal Susceptibility Testing between Conventional Method (E-Test) and Automated Method (Vitek-2) Out of Total 50 Isolates

Candidaspecies	**Sensitive isolates**		**Resistant isolates**	
Total Candida species (50) by Conventional method	**Conventional methods (E-test)**	**Automated method (VITEK-2)**	**Conventional methods (E-test)**	**Automated method (VITEK-2)**
***Candida tropicalis* (21)**	07	22	**14**	00
***Candida albicans* (14)**	09	09	**05**	00
***Candida parapsilosis* (11)**	07	09	**04**	00
Candida famata	00	06	00	00
***Candida guilliermondii* (01)**	01	02	00	**01**
***Candida glabrata* (02)**	01	00	**01**	00
***Candida kefyr* (01)**	01	00	00	00
Candida lusitaniae	00	00	00	**01**

Fig.1 CHROM Agar Inoculation for Different *Candida species* Showing- Light Green Colour (*C.albicans*); Bluecolour (*C.Tropicalis*) and Cream to Pale Pink Colour (*C.parapsilosis*)

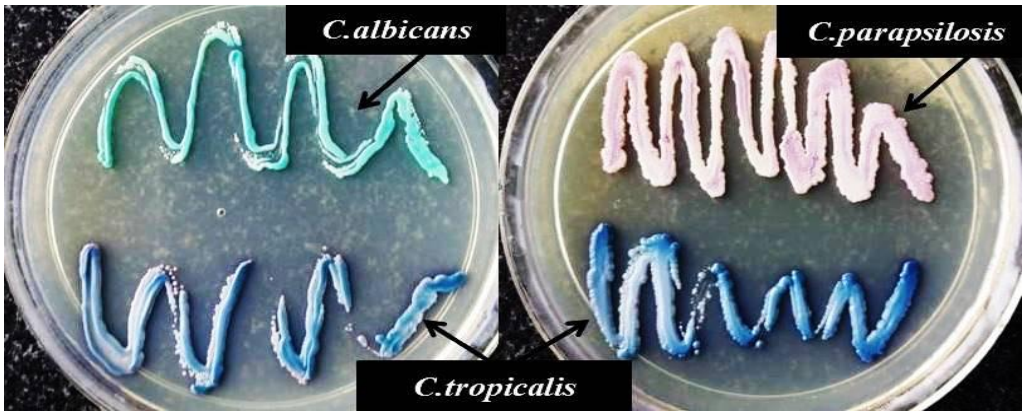


Fig.2 Sugar Assimilation Test (Indicator Bromocresol Purple) by Pour Plate Method for Eight Different Sugars for *C.albicans*. Yellow Discoloration and Growth Around Glucose, Maltose, Sucrose and Trehalose Indicates a Positive Assimilation Test

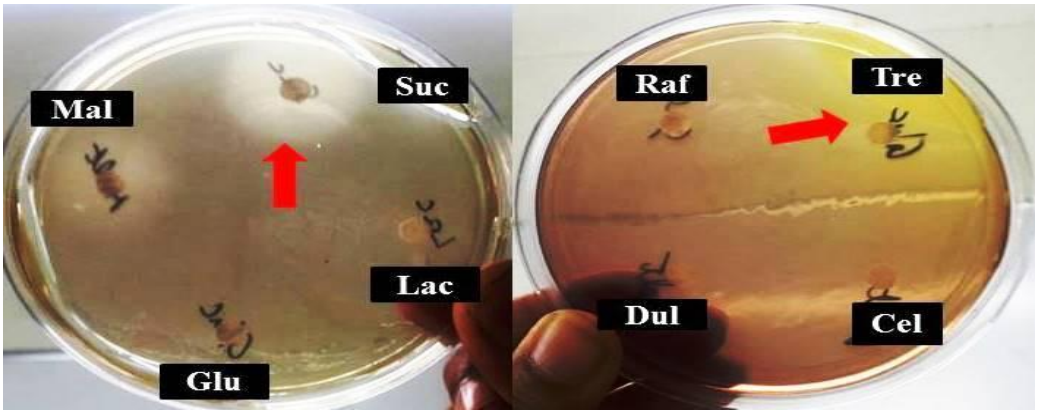


Fig.3 Cornmeal Agar with Tween-80 Inoculation for *Candida species*: 1. *C.albicans* Showing Branching Pseudo Hyphae with Bunch of Blastoconidia and Terminal Single Chlamydospores. 2. *C.glabrata*- Budding Yeast without Pseudo Hyphae. 3. *C.parapsilosis*- Abundant Branched Pseudo Hyphae (Tree Like Pattern) with Few Blastoconidia. 4. *C.tropicalis*- Long Pseudo Hyphae with Numerous Ovoid blastoconidia

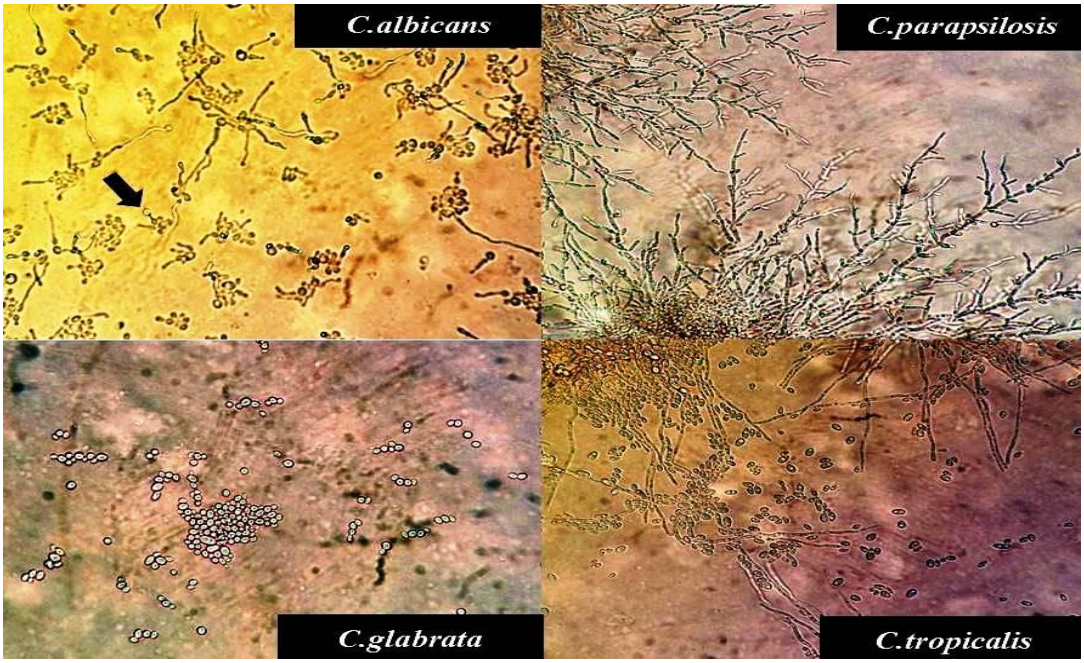


Fig.4 E-Test Method for AFST: Sensitive to Fluconazole and Amphotericin B (left) and Voriconazole (right)

Fig.5 E-Test Method for AFST: Exhibiting Resistance to Fluconazole and Voriconazole

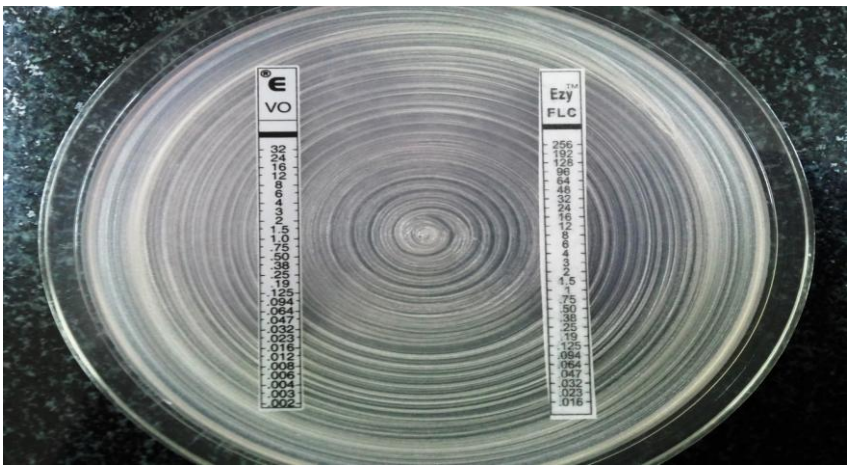


Fig.6 Distribution of Various Clinical Samples

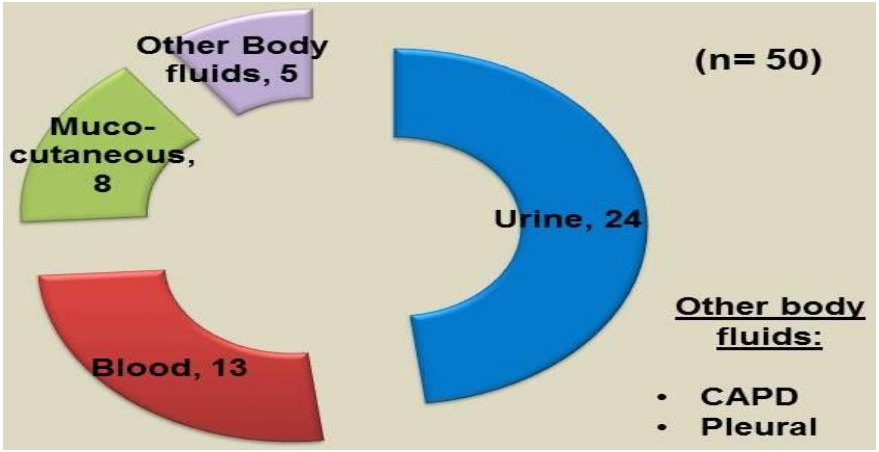
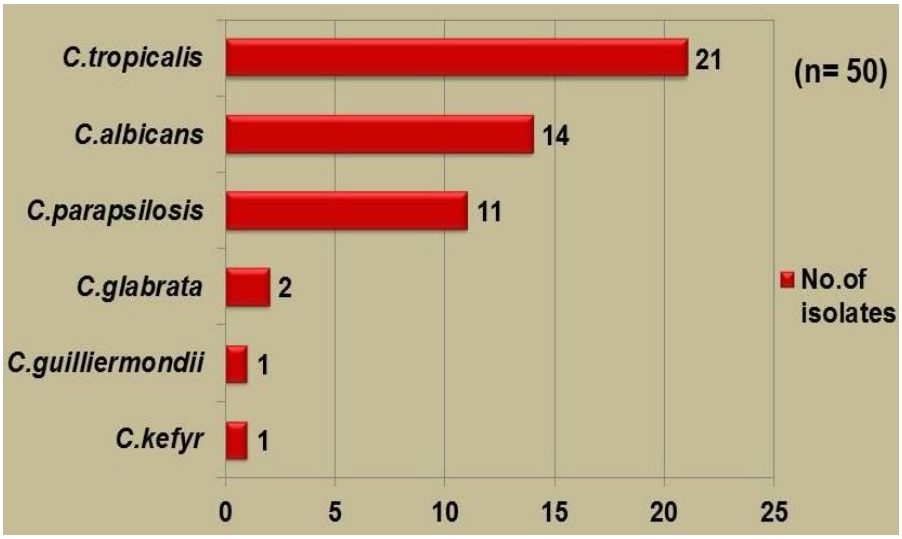


Fig.7 Distribution of Different *Candida spp* by Conventional Phenotypic Methods



All the 50 isolates were subjected to anti-fungal susceptibility testing, out of which VITEK-2 identified only 4% (02 isolates) as resistant to fluconazole (*C.guilliermondii* and *C.lusitaniae*). By E-test method, 48% (24 isolates) of isolates were found to be resistant to fluconazole and voriconazole and all isolates were sensitive to amphotericin B (16).Out of these 24 resistant isolates, 14 were *C.tropicalis*, 05 were *C.albicans*, 04 were *C.parapsilosis* and 01 was *C.glabrata*. A total of 4% (02 isolates) of isolates which showed fluconazole resistance by VITEK-2 were sensitive by E-test. Fluconazole and voriconazole resistance by E-test was 48%(24 isolates) whereas VITEK-2 identified all of them as sensitive. There was less than chance agreement between VITEK-2 and E-test for antifungal susceptibility testing.

A study conducted by Charles *et al* reported the remarkable increase of inherently fluconazole resistant non-albicans Candida recently. They suggest that newer azoles like posaconazole and echinocandins are suitable alternatives (17).The fluconazole resistant *C.albicans*, *C.krusei* and *C.glabrata* were found to be susceptible toVoriconazole in-vitro in a study by Barry AL *et al* (1996) and Ruhnke M *et al* (1997)(18,19). In this study, maximum fluconazole and voriconazole resistance was seen in *C.tropicalis* followed by *C.albicans* and others. These results are in concurrence with the results of other studies like Barry AL *et al* (1996), Ruhnke M *et al* (1997) and Barchiesi F *et al* (2000) (18,19,20).In the present study, there was no isolate which was found to be resistant to amphotericin B, whereas some studies have reported a resistance of 6.9% byCapoor MR*et al* (2005) and 2.5 to 16.3% by Yang YL *et al* (2005)(11,21).

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.tropicalis* was most common species. We conclude that E-test method can be used as a simple method for antifungal susceptibility testing. Moreover, we cannot rely only on VITEK-2for identification and antifungal susceptibility testing. This study recommends the identification of *Candida* species by conventional methods and

antifungal susceptibility testing by E-test method, because the treatment varies accordingly, due to the difference in antifungal susceptibility profile.

References

1. Jacqueline M, Achkar, Bettina C*et al*. Candidal Infections of the genitourinary tract. *Clinical Microbiology Review*2010; 23(2):253-72.
2. Falgas ME, Apostolou KE, Pappas VD. Attributable mortality of Candidaemia: A systemic review of matched cohort and case control study. *European Journal of clinical microbiology Infectious Disease* 2006; 25:419-25.
3. Goodman JL, Winston DJ, Greenfield RA, Chandrasekar PH, Fox B, Kaiser H, *et al*. Acontrolled trial of fluconazole to prevent fungal infections in patients undergoingbone marrow transplantation. *New England Journal of Medicine*1992; 326:845–851.
4. Redding S, Smith J, Farinacci G, Rinaldi MG, Fothergill AW*et al*. Development of resistance tofluconazole among isolates of *Candida albicans* obtained during treatment of oropharyngeal candidiasis in AIDS: documentation by *in vitro* susceptibility testing and DNA subtype analysis. *Journal of Clinical Infectious Disease* 1994; 18:240–242.
5. Adhikary R, Joshi S. Species distribution and antifungal susceptibility of Candidaemia at multi super- speciality center in southern India. *IJMM*2011;29:309-11.
6. Chander J. A textbook of medical mycology (Mehta publishers, Nariana II, New Delhi, India). 3rd edition 2009; 266-83.
7. Topley WWC, Wilson CS. Candidiasis, in Topley Wilson's Medical mycology. Chapter 30:579- 13.
8. Rani R, Mohapatra NP, Mehta G, Randhawa VS. Changing trends of *Candida species* in tertiary north Indian hospital. Indian *Journal of Medical Microbiology*2002;20:42-4.
9. Agarwal J, Bansal S, Malik GK. Trends in neonatal septicemia. Emergence of non-albicans Candida, *Indian J Paediatrics* 2004; 41:712-15
10. Chakrabarti A, Mohan B, Shrivastava SK, Marrak RSK, Ghosh A, Ray P. Change in distribution of Antifungal susceptibility of *Candida species* isolated from Candidaemia cases from tertriary centre during 1996-2000. *Indian Journal of Medical Research*2002;116:5-12.
11. Capoor MR, Nair D, Deb M, Verma PK, Srivastva L, Aggarwal P: Emergence of non-albicans Candida species and antifungal resistance in a tertiary care hospital. *Japan Journal of Infectious Disease*2005; 58:344-48.
12. Krcmery V *et al*. non albicans *Candida* species causing fungaemia: pathogenicity and antifungal resistance. *Journal of Hospital Infections*2002; 50 (4):243-260.
13. Wingard JR. Importance of Candida species other than C.albicans as pathogens in oncology patients. *Clinical Infectious Disease*1995; 20:115-25.
14. Jensen RH, Arendrup MC. Characterization of a previously overlooked pathogen and its unique susceptibility profile in comparison with five related species. *Journal of Clinical Microbiol*2011;49:549–556.
15. Castanheira M, Woosley LN, Diekema DJ, Jones RN and Pfaller MA. *Journal of Clinical Microbiology*2013; 51(1):117.
16. Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*; Approved Standard.

CLSI document M27-S4. Wayne, Pennsylvania, USA. 2012.
17. Charles PE, DoiseJM, Quenot JP, Aube H, Dalle F *et al*.Candidaemia in critically ill patients: difference of outcome between medical and surgical patients. *Intensive Care Medicine*. 2003; 29:2162-2169.
18. Barry AL and Brown SD. *In vitro* studies of two triazole antifungal agents (voriconazole and fluconazole) against Candida species. *Antimicrobial agents and chemotherapy*1996; 40:1948-9.
19. Ruhnke M, Schmidt-Westhausen A and Trautmann M. In-vitro activities of voriconazole against fluconazole susceptible and resistant C.albicans isolates from oral cavities of patients with HIV infection. *Antimicrobial agents and chemotherapy*1997; 41:575-7.
20. Barchiesi F, Arzeni D, Fothergill AW, Di Francesco LF, Caselli F *et al*. *In vitro* activities of the new antifungal triazole SCH 56592 against common and emerging yeast pathogens. *Antimicrob Agents Chemother*2000; 44:226–229.
21. Yang YL, Li SY, Cheng HH and Lo HJ. Susceptibilities to amphotericin B and fluconazole of Candida species in TSRAY. Diagnostic Microbiology Infectious Disease 2005; 51:178-83.
22. Graph Pad Software Quickcalcs [home page on internet]. No date [cited 2014 Nov 20]. Available from: http://graphpad.com/ quickcalcs/ kappa1. cfm.

How to cite this article:

Prasanna, S., Partha Roy, Naveen Grover, Puneet Bhatt, Dudhat Vaibhav Laxmikant Rao and Nikunja Kumar Das. 2016. A Comparative Analysis on Identification and Antifungal Susceptibility Profile of Candida Isolates Using Conventional and Automated Techniques *Int.J.Curr.Microbiol.App.Sci.* 5(3): 579-589.doi: http://dx.doi.org/10.20546/ijcmas.2016.503.067