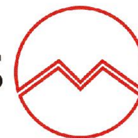
Websites:
http://www.sciencepub.net/nature
http://www.sciencepub.net

Emails:
naturesciencej@gmail.com
editor@sciencepub.net

Nature and Science

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher



Identification of Yeasts by Using matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (Maldi-Tof Ms) In comparison to Other Detection Methods in Microbiological Samples

Emtethal El-Sayed El- Kholy[1], Asmaa Abd El-Salam El-Madbouly[1], Ghada El-Sayed Amr[2] and Sara Ahmed Tahoun[1]

[1]Department of Clinical Pathology, Faculty of Medicine for Girls- Alazhar University, Cairo, Egypt.
[2]Department of Clinical Pathology, Faculty of Medicine, Al-Zagazig University, Cairo, Egypt.
dr.saratahoun@gmail.com

Abstract: Background: Identification of yeast species still relies primarily on culture and phenotypic methods, which is labour-intensive and time-consuming. The rising frequency of hospital acquired yeast infections underlines the need for an accurate, rapid and simple identification tool at the species level. **Objective**: The aim of this study was to compare the identification performance of yeast species with phenotypic methods as chromogenic Candida Agar (CAN2) and Vitek 2, versus identification with matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)- Mass spectrometry. **Methods**: From 500 clinical samples provided to the microbiology laboratory of AL-Zahraa University hospital, Cairo, Egypt, 50 yeast isolates were randomly selected and subjected to germ tube test, Culture on Chrom ID™ Candida Agar (CAN2), Vitek 2 and MALDI-TOF MS. **Results:** Out of 50 yeast isolates, 19 isolates were obtained from urine (38%), 14 sputum (28%),7blood isolates (14%), 5 throat isolates (10%), 2 cervical swab isolates (4%), 2 chest tube isolates (4%), 1 endotracheal tube isolate (2%), 1wound swab isolate (2%) and 1 ear swab isolate (2%). Overall, 96% yeast identifications were in agreement between the MALDI TOF/MS and conventional identification at the genus level, and 82% at species level**. Conclusion**: This study confirms the role of MALDI-TOF MS in clinical settings for the rapid and accurate identification of yeast.

[Emtethal El-Sayed El- Kholy, Asmaa Abd El-Salam El-Madbouly Ghada El-Sayed Amr and Sara Ahmed Tahoun. **Identification of Yeasts by Using matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (Maldi-Tof Ms) In comparison to Other Detection Methods in Microbiological Samples.** *Nat Sci* 2020;18(4):12-19]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 3. doi:10.7537/marsnsj180420.03.

Key Words: MALDI-TOF MS, Vitek 2, Yeast, Identification.

1. Introduction

The incidence of invasive fungal infections has steadily increased in the last decade, with yeasts dominating in numbers over filamentous fungi ***(Pfaller and Diekema, 2010).***

Rapid species identification can facilitate early institution of suitable antifungal therapy and expedite recovery in high-risk patients. This is even more pertinent for emerging rare yeasts resistant to multiple antifungal agents ***(Miceli et al., 2011).***

Conventional phenotypic methods, although useful for routine identification of common Candida species, are time consuming and inept at identifying rare yeasts. In contrast, identification by molecular techniques such as rDNA sequencing offers a rapid and reliable alternative ***(Cornet et al., 2011)***.

However, despite its advantages, it entails a steep learning curve, need for expertise, higher costs and relatively longer run time than other faster techniques. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is rapidly becoming popular for routine identification of clinical pathogens ***(Cherkaoui et al., 2010).***

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a molecular analytic tool that has been used extensively for protein analysis and was applied recently to clinical microbiology. MALDI-TOF MS shows rapid turnaround time, low sample volume requirements, and modest reagent costs. Peptide or protein mass-to-charge (*m*/*z*) values form mass spectral peaks, indicating the molecular masses and charge densities of components present in a biological sample. These spectra can generate pathognomonic patterns that provide unbiased identifications of particular species and even genotypes within species ***(Cherkaoui et al., 2010).***

The aim of this study was to compare the identification performance of yeast species with phenotypic methods as germ tube test, chromogenic Candida Agar (CAN2) and Vitek 2, versus identification with matrix-assisted laser

desorption/ionization-time of flight (MALDI-TOF)-Mass spectrometry.

2. Material and Methods

Setting:

This study was carried out in the Clinical microbiology laboratory of the clinical pathology department, Al Zahra University Hospital, AL-Azhar University, Cairo, Egypt, during the period from April 2016 to April 2018.

Yeast isolates:

From 500 clinical samples provided to the microbiology laboratory, 50 yeast isolates were randomly selected, after routine culture on blood agar, Mac Conkey's agar and Sabouraud dextrose agar (SDA). These clinical samples included: urine, sputum, blood, wound swabs, vaginal swabs and throat swabs.

Methods:

The isolated 50 yeast samples were then subjected to:

I- Secondary subculture on SDA and gram staining that revealed the presence of gram positive budding yeast cells with pseudohyphae.

II- Germ tube test

The germ-tube test was performed according to standard methods using pooled human serum and incubation at 36.7C for 3 h.

III- Cultureon Chrom ID™ Candida Agar (CAN2)

All isolates were inoculated by streakingon Chrom ID™ Candida Agar (CAN2) (biome'rieux, Marcy l'Etoile, France) after inoculation, the plates were immediately incubated at 37°C in aerobic conditions and in the dark. The cultures were generally examined after 24, 48 hours of incubation and the color of the colonies was observed:

• Pale blue to dark blue: characteristic of Candidaalbicans.

• Pink: characteristic of Candida tropicalis, Candida lusitaniae and Candida kefyr.

Identification of the microorganism isolated must be followed by biochemical and/or immunological tests.

• Creamy-white: no predictive value.

IV- VITEK 2 (biome'rieux, Marcy l'Etoile, France) and ID-YST card:

The VITEK ID-YST card consists of 64 wells with 47 fluorescent biochemical tests. Inocula were adjusted to a no. 2 McFarland standard using an ATB 1550 densitometer (bioMérieux, Marcy-l'Etoile, France) by suspending the yeast cells in 0.45% aqueous NaCl. The integrated VITEK 2 instrument automatically filled, sealed and transferred the cards into an incubator. Every 15 min the cards were automatically subjected to a fluorescence measurement. Each profile was interpreted according to a specific algorithm. After an incubation period of 15 h, the profile result was compared to the ID-YST database, which led to the final identification of the microorganism.

V- MALDI-TOF MS (Marcy l'Etoile, France) biomerrieu

A yeast colony (typically single) is "picked" from a culture plate and placed onto a ground steel MALDI target plate. Subsequently, each sample was overlaid with 1-2ul of matrix, which consisted of a saturated solution of _-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid, and air dried at room temperature. Then the plate is placed in the ionization chamber of the mass spectrometer for analysis. A mass spectrum is generated and automatically compared against a database of mass spectra by the software resulting in identification of the organism.

Statistical Methods:

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science **(IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).** Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Frequency and percentages were used to describe non-numerical data. **Chi-Square test** was used to examine the relationship between two qualitative variables. **Fisher's exact test:** was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. The kappa statistic was calculated to estimate agreement between the methods. The strength of agreement of kappa coefficients was guided by the boundaries suggested by Landis and Koch [1]. Values less than 0 indicate "poor" reliability, 0-0.20 is "slight" reliability, 0.21-0.40 is "fair" reliability, 0.41-0.60 is "moderate" reliability, 0.61-0.80 is "substantial" agreement, 0.81-1.00 "excellent" or "almost perfect" agreement.

N.B: p is significant if <0.05 at confidence interval 95%.

3. Results

The present study was conducted on 50 yeast isolates from 500 clinical isolates sent to microbiology lab. Al Zahraa University Hospital for culture and sensitivity during the period from April 2016 to April 2017.

The isolates were derived from 50 clinical samples taken from 50 patients, 14 of them were males (28%) and 36 were females (72%).

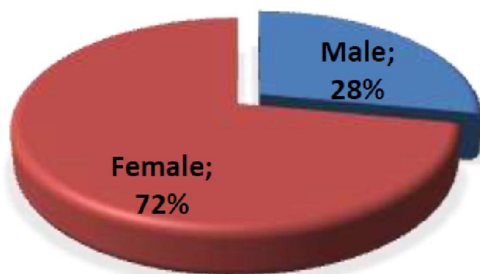


Figure (1): Gender of patients involved in the study.

Twenty four patients were immune competent (48%) and 26 cases were immune compromised (52%) according to the mentioned criteria of immune compromization.

The type of clinical specimen sources of the studied yeast isolates were shown in table 1.

Table (1): The distribution of yeast isolates according to the source of specimen

	Total isolates (N=50)	
	N	**%**
Urine	19	38
Sputum	14	28
blood culture	7	14
Throat swab	5	10
Cervical swab	1	2
chest tube	1	2
endotracheal tube	1	2
wound swab	1	2
ear swab	1	2

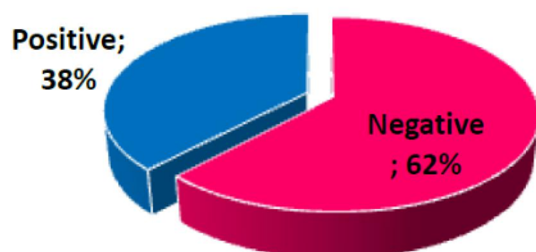


Figure (2): Germ tube test results in all studied isolates.

Germ tube test results:

Most isolates showed negative germ tube test (62%), while (38%) showed positive test (fig.2).

1. **Chrom ID™** Candida Agar (CAN2) results:

In the current study, 22 of yeast isolates showed blue color for C. albicans (44%), 13 showed pink color for C tropicalis. C lusiteniae. Ckefyr (26%) and 15 isolates showed white color had no predictive value (30%).

2. Vitek 2 ID- YST results:

Candida albicans was identified in 19 isolates, non albicans candida species were identified in 27 isolates. Non candida species were identified in 3 isolates (**Trichosporon asahii, Geotrichum Capitatum** and **Cryptococcus Laurentii**) and 1 isolate was unidentified.

The degree of isolate discrimination by Vitek 2 was illustrated in table 2.

Table (2): Level of yeast discrimination by Vitek 2 ID-YST cards

	Total isolates N=50	
	N	**%**
Unidentified	1	2
Low discrimination	6	12
Acceptable	1	2
Good	4	8
very good	11	22
Excellent	27	54

3. **MALDI-TOF results:**

Forty seven isolates showed candida (19 isolates were C. albicans, 10 were C. Glabrata, 7 were C. Tropicalis, 5 were C. Parapsilosis, 1 was C. Lusitaniae, 3 were C. Krusei, 1 was C. Kefyr and 1 was C. Guilliermondii) and 3 isolates were non candida yeasts (one isolate was Trichosporon asahii and two isolates was Geotrichum Capitatum).

Agreement between MALDI- TOF and other phenotypic methods for identification of candida and non-candida yeast species was shown in table 3.

Table (3): Agreement between CHROM agar, **Vitek 2 ID- YST** and MALDI-TOF MS results for candida identification from other yeasts

		Chromagar		***K***	**Agreement**
		Non candida yeasts	**Candida**		
MALDI-TOF MS	**Non candida yeasts**	2	1	0.136	Slight
	Candida	13	34		
		Vitek 2 ID- YST			
MALDI-TOF MS	**Non candida yeasts**	1	2	0.485	Moderate
	Candida	0	47		

Agreement between MALDI- TOF and other phenotypic methods for candida albicans identification from non albicans Candida was shown in table 4.

Performance of MALDI-TOF MS in comparison to Vitek 2 ID-YST, CHROMagar results, for identification of candida and non-candida yeast species.

Table (4): Agreement between germ tube tests, **Chrom agar, Vitek 2 ID- YST** and MALDI-TOF MS results for candida albicans identification from candida non albicans.

		Germ tube test		***k***	**Agreement**
		Candida non albicans	**C. albicans**		
MALDI-TOF MS	**Candida non albicans**	31	0		
	C. albicans	0	19	1	Perfect
		Chromagar			
		Candida non albicans	**C.albicans**		
MALDI-TOF MS	**Candida non albicans**	28	3		
	C.albicans	0	19	0.876	Very good
		Vitek 2 ID- YST			
		Candida non albicans	**C.albicans**		
MALDI-TOF MS	**Candida non albicans**	30	1		
	C.albicans	1	18	0.915	Very good

Table (5): Performance of Vitek 2 ID-YST, Chrom ID™ Candida Agar (CAN2) results, in comparison to MALDI-TOF MS for identification of candida and non-candida yeast species

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
Chrom A gar	97.1	13.3	72.3	66.7	72.0
Vitek 2 ID- YST	95.9	100.0	100.0	33.3	96.0

Table (6): Performance of germ tube, Vitek 2 ID-YST, CHROM agar media, in comparison to MALDI-TOF MS for identification of candida abicans and candida non albicans species

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
Germ tube	100	100	100	100	100
Chrom Agar	86.4	100	100	90.3	94
Vitek 2 ID-YST	94.7	96.8	94.7	96.8	96

Concordance between methods:

Table (13): Concordance of species identification by chrom gar, Vitek2 and MALDI-TOF

	Concordance between methods					
	ChomAgar-Vitek2-MT		**ChromAgar_MT**		**Vitek2-MT**	
	N	**%**	**N**	**%**	**N**	**%**
Total	26	52	28	56	40	80
C. albicans	18	36	19	38	18	36
C. Tropicalis	6	12	7	14	6	12
C. Lusitaniae	1	2	1	2	1	2
C. Kefyr	1	2	1	2	1	2
C. Glabrata	0	0	0	0	5	10
C. Parapsilosis	0	0	0	0	5	10
C. Krusei	0	0	0	0	3	6
Trichosporon asahii	0	0	0	0	1	2

Table (8): Distribution of candida species according to MALDI-TOF, Vitek II, chromagar, germ tube test for identification of *Candida* species from agar plates

Germ tube test	ChromAgar	Vitek II	MT	N	%
Positive (n=19)	**Blue**	**C.albicans**	**C.albicans**	18	36
		C. Famata	**C.albicans**	1	2
Negative (N=31)	**blue**	**C. Glabrata**	**C. Glabrata**	1	2
		C. Dubliniensis	**C. Glabrata**	1	2
		Trichosporon asahii	**Trichosporon asahii**	1	2
	Pink	**C. Glabrata**	**C. Glabrata**	1	2
		C. Tropicalis	**C. Glabrata**	1	2
			C. Tropicalis	6	12
		C. Famata	**C. Tropicalis**	1	2
			C. Guilliermondii	1	2
		C. Dubliniensis	**C. Glabrata**	1	2
		C. Lusitaniae	**C. Lusitaniae**	1	2
		C. Kefyr	**C. Kefyr**	1	2
	White	**C.albicans**	**C. Glabrata**	1	2
		C. Glabrata	**C. Glabrata**	3	6
		C. Parapsilosis	**C. Parapsilosis**	5	10
		C. Krusei	**C. Krusei**	3	6
		Saprochaete Capitata	**Geotrichum Capitatum**	1	2
		Cryptococcus Laurentii	**C. Glabrata**	1	2
		Unidentified	**Geotrichum Capitatum**	1	2

4. Discussion

Candida species represent the fourth most common pathogen isolated from blood cultures, and infections are associated with a high mortality. In addition to Candida species, several other yeasts such as Geotrichum spp., Trichosporon spp. and Cryptococcus spp. are increasingly observed in immunosuppressed patients ***(Birrenbach et al., 2012).***

These species are often resistant to several antifungal drugs, including an intrinsic resistance to the echinocandins, which are recommended in several guidelines as the first-line therapy for candidaemia in most patient groups ***(Ullmann et al., 2012).***

The correct and fast identification of fungal pathogens from clinical specimens and from patients' environments, especially in outbreak situations, is of major concern for optimal patient management and the implementation of effective measures for disease control ***(Marklein et al., 2009).***

As an alternative to the conventional and biochemical methods, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) has emerged as a rapid and accurate tool for identifying pathogens, including bacteria, mycobacteria, moulds and yeasts ***(Pulcrano et al., 2013).***

In the present study we evaluated the different phenotypic techniques for the identification of 50 yeast isolates that were randomly selected from clinical specimens referred to the microbiology laboratory of Al Zahraa University Hospital for routine culture and sensitivity during the period from April 2016 to April 2017.

Among these 50 isolates, 14 were derived from males (28%) and 36 were derived from females (72%). This significant higher isolation of yeasts from female patients was similar to other studies by **Meena et al., 2018**, **Bhattacharjee 2016**, and **Dutta et al., 2015**.

In the present study, 19 of 50 yeast isolates were derived from urine (38%), followed by14 from sputum (28%), 7blood isolates (14%), 5 throat isolates (10%), 2 cervical swab isolates (4%), 2 chest tube isolates (4%), 1 endotracheal tube isolate (2%), 1wound swab isolate (2%) and 1 ear swab isolate (2%).

This was in concordance with the study performed by Meena et al., 2018 where a total of 317 Candida isolates from various clinical sources were isolated. Out of these,93 Candida isolates were obtained from urine (29.3%), 74 (23.30%) from blood, 58 (19.0%) from high vaginal swab. Similar findings were obtained by **Sachin et al., 2014 and Mnge et al., 2017.**

However, different sample distrubution was reported by Souza et al. as 66 of 89 isolates of Candida spp. were obtained from blood followed by urine, ascetic and synovial fluids ***(Souza et al., 2015).***

In the present study we identified 56% non albicans Candida isolates which was more than Candida albicans isolates 38%. **Meena et al., 2018** also identified higher non albicans Candida isolates (57.73%) than Candida albicans isolates (42.27%).

Similarly, a study by **Rachna**,**2016**showed increasing trend of non albicans Candida isolation (59.13%) than Candida albicans (40.87%).

In the present study all isolates of C. albicans gave positive GTT with 100% sensitivity and specificity for differentiating candida albicans from non albicans candida species.

Consistent with our GTT results, some studies reported sensitivity and specificity rates ranging from 92 to 98.8% and 99 to 100%, respectively ***(El-sayed and Hamouda 2007).***

Our GTT results were in agreement with those obtained by **Souza et al., 2015** that showed sensitivity of (89.3%), and specificity of (96.7%) and reported that the germ tube test is the gold standard of laboratories for the identification of *C. albicans*, because besides being a quick and simple technique, among all *Candida* species, this is the only method able to detect germ tube formation in the presence of human or animal serum when samples are incubated at 37 °C for two to four hours. However, they recommended that additional tests are required ***(Souza et al., 2015).***

Some limitations of GTT are that it is time consuming and laborious as 100 yeast cells should be examined before the test is declared negative. Also, experience is needed for correct recognition of germ tubes ***(Freydière and Guinet 1997).*** Misinterpretation of elongated blastoconidia as germ tube, absence of germ tube production in some strains of C. albicans, and health hazards of handling pooled sera ***(Elfeky et al., 2016).***

In the present study the performance of CAN2 agar in isolation of candida from other yeasts was of 95.9% sensitivity and 100% specificity which were in agreement with the study performed by Elfeky et al., 2016 where 90.5% of Candida isolates were correctly assigned into the three groups of yeasts identified by CAN2 agar.

Elfeky et al., reported that Chromogenic culture media are very useful for the diagnosis of Candida but their main limitation is the low discrimination power among Candida species ***(Elfeky et al., 2016).*** Which was concordant with the results of the current study as colonies of NAC species can give the same color on chromogenic media; Pink colonies characteristic of *Candida tropicalis*, *Candida lusitaniae* and *Candidakefyr*. While, Creamy-white colonies have no predictive value. So in the light of that, Identification of the microorganism isolated must be followed by additional tests.

In the present study, Vitek 2 results for identification of yeasts and yeast-like organisms; showed sensitivity 94.7%, and 96.8 % specificity when compared to MALDI-TOF MS which was considered the gold standard in this study. Vitek-2 identified 39 out of 50 yeast isolates (78%) correctly, 9 (18%) were misidentified, 1 (2%) was identified with low discrimination, and 1 (2%) was unidentified.

Similar findings were obtained by **Graf et al., 2002** when the VITEK 2 system was evaluated for rapid identification of yeasts and yeast-like organisms in comparison to the ID 32C system as a gold standard. The VITEK 2 system showed better performance for the correct identification with or without additional tests (92.1 and 87.6%, respectively).

In the present study with Vitek-2 ID system, most problems were encountered with the identification of Geotrichumcapitatum (n=2, 4%), which was unidentified by Vitek-2 ID system in one isolate, C. glabrata (n=10, 20%): 5 isolates were misidentified and 1 isolate was identified with low discrimination, C. albicans (n=19, 38%): 1 isolate was misidentified, C. guillermondii (n=1, 2%): 1isolate was misidentified and C. tropicalis (n=7, 14%): 1 isolate was misidentified.

In the study performed by **Kaur et al., 2016** with Vitek-2 ID system, the identification of C. albicans was problematic as 11 isolates were misidentified and two isolates were identified with low discrimination.

In the present study, Cryptococcus laurentii (n =1, 2 %) and C. famata (n =3, 6%) were the common species most misreported species by the Vitek YST-ID card. C. laurentii and C. famata were not found in this study as confirmed by MALDI-TOF MS. The low discrimination report of Cryptococcus laurentii by Vitek-2 ID system was found to be C. glabrata by MALDI-TOF MS with 99.9% confidence level. The low discrimination report of C. famata/C. guilliermondii by Vitek-2 ID system was found to be C. guilliermondii by MALDI-TOF MS with 99.9% confidence level. This was in agreement with the study by Nath et al.,2017 with Vitek-2 ID system as Cryptococcus laurentii (n = 7, 26.9%), C. parapsilosis (n = 6, 23.07%), C. rugosa (n = 4, 15.38%) and C. famata (n = 4, 15.38%) were the common species misreported by the Vitek YST-ID card. C. laurentii and C. famata were not found in that study as confirmed by ITS sequencing.

In the present study MALDI-TOF MS proved to be a rapid and reliable procedure for the accurate identification of pathogenic yeast strains and required minimal hands-on time or time for the interpretation of the results. The test procedure was generally completed within 10 min per isolate, starting from single yeast colonies on the agar plate.

Overall, MALDI-TOF MS identified all tested yeast isolates, and the spectra for the appropriate reference strains were present in the database.

All identified yeast isolates by Vitek-2 system with low discrimination level were identified with high confidence level (99.9%) by MALDI-TOF MS.

Our findings confirm the high agreement (k=0.915) for yeast identification using the standard, phenotypic laboratory methods and mass spectrometry technique. Although our high agreement is based on the analysis of 50 clinical, patient-derived strains only, these results remain in accordance with previous observations on larger numbers of clinical isolates **(Van Veen et al., 2010; Sow et al., 2015).**

Conclusion

In summary, conventional methods of fungal identification are very useful in clinical microbiology laboratories. Identification by conventional microbiology methods takes at least 16 hours, if a culture is positive. The introduction of MALDI-TOF MS in the microbiology laboratory could mean a radical change in the identification accuracy, reducing time (10 minutes per a single isolate). Provided that appropriate standard operating procedures and comprehensive quality-controlled databases with the spectra for human pathogens are available.

References:

1. Ghosh, A K, Paul, S, Sood, P, et al (2015): Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts causing bloodstream infections. Clinical Microbiology and Infection; 21: 372-378.
2. Alam MZ, Alam Q, Jiman-Fatani A, et al (2014): Candida identification: a journey from conventional to molecular methods in medical mycology. World J Microbiol Biotechnol; 30(5):1437-51.
3. Bhattacharjee P. (2016): Epidemiology and antifungal susceptibility of Candida spp. in a tertiary care hospital, Kolkata. India Curr Med Mycol.;2 (2): 20-27.
4. Birrenbach T, Bertschy S, Aebersold F, et al (2012): Emergence of Blastoschizomycescapitatus yeast infections, Central Europe. Emerg Infect Dis; 18: 98-101.
5. Boyanton BL, Luna RA, Fasciano KG et al (2008): DNA pyrosequencing-based identification of pathogenic Candida species by using the internal transcribed spacer 2 region. Arch. Pathol. Lab. Med. 132:667-674.
6. ElFeky DS, Gohar NM, El-Seidi EA, et al (2016): Species identification and antifungal susceptibility pattern of Candida isolates in cases of vulvo-vaginal candidiasis. Alexandria Journal of Medicine; 52; 269-277.
7. El-sayed H and Hamouda A (2007): Candida albicans causing vulvovaginitis and their clinical response to antifungal therapy, Egypt J Med Microbiol;16(1):53-62.
8. Graf B, Adam T, Zill E, et al (2000): Evaluation of the VITEK 2 System for Rapid Identification of Yeasts and Yeast-Like Organisms, Journal Of Clinical Microbiology, 38(5); 1782-1785.
9. Hata DJ, Hall L, Fothergill AW et al (2007): Multicenter Evaluation of the New VITEK 2 Advanced Colorimetric Yeast Identification Card, Journal Of Clinical Microbiology, 45(4); 1087-1092.
10. Kaur R, Dhakad MS, Goyal R, et al (2016): Identification and Antifungal Susceptibility Testing of Candida Species: A Comparison of Vitek-2 System with Conventional and Molecular Methods, J Glob Infect Dis; 8(4): 139-46.
11. Kim D, Shin WS, Lee KH, et al (2002): Rapid differentiation of Candida albicans from other Candida species using its unique germ tube formation at 39 ◦C, Research Article, Yeast; 19: 957-962.
12. Kim TH, Park BR, Kim HR, et al (2010): Candida dubliniensis screening using the germ tube test in clinical yeast isolates and prevalence of C. dubliniensis in Korea. J Clin Lab Anal 24: 145-148.
13. Lo´ pez-Martı´nez R (2010): Candidosis, a new challenge. Clinics Dermatol;28:178-84.
14. Marklein G, Josten M, Klanke U, et al (2009): Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J ClinMicrobiol 47(9): 2912-2917.
15. Massonet C, Eldere JV, Vaneechoutte M, et al (2004): Comparison of VITEK 2 with ITS2-Fragment Length Polymorphism Analysis for Identification of Yeast Species, Journal Of Clinical Microbiology, 42 (5); 2209-2211.
16. Mattei AS, Alves SH, Severo CB, et al (2014): Use of Mueller-Hinton broth and agar in the germ tube test. Rev Inst Med Trop Sao Paulo;56(6):483-5.
17. Meena K, Gandhi S, Jain SB et al (2018): Phenotypic characterization and antifungal susceptibility pattern of candida species in tertiary care center. Indian journal of applicated research;8(4):2249-555.
18. Melhem MS, Bertoletti A, Lucca HR, et al (2014): Use of the VITEK 2 system to identify and test the antifungal susceptibility of clinically relevant yeast species. Braz J Microbiol, 44:1257-66.

19. Nath R, Bora R, Borkakoty B, et al (2017): Clinically relevant yeast species identified by sequencing the internal transcribed spacer region of r-RNA gene and Vitek 2 compact (YST card) commercial identification system: Experience in a Tertiary Care Hospital in Assam, Northeast India, Indian J Med Microbiol;35 (4):588-92.
20. Neppelenbroek KH, Urban VM, Silva S, et al (2014): Identification of Candida species in the clinical laboratory: a review of conventional, commercial, and molecular techniques, REVIEW ARTICLE, Oral Diseases; 20; 329-344.
21. Mnge P, Okeleye BI, Vasaika SD et al (2017): Species distribution and antifungal susceptibility patterns of Candida isolates from a public tertiary teaching hospital in the Eastern Cape Province, South Africa. Brazilian Journal of Medical and Biol Research.; 50(6):1-7.
22. Pfaller MA and Diekema DJ (2010): Epidemiology of invasive mycoses in North America. Crit Rev Microbiol 36(1):1-53.
23. Pulcrano DV, Iula A, Vollaro A, et al (2013): Rapid and reliable MALDI-TOF mass spectrometry identification Candida non-albicans isolates from bloodstream infections. J. Microbiol. Methods; 94: 262-266.
24. Rachna M and Anupama SW (2016): Evaluation of Hi Chrome Candida Differential Agar for Species Identification of Candida Isolate from Various Samples. International Journal of Contemporary Medical Research; 3: 1219-1222.
25. Roopa C and Biradar S (2015): Isolation of Candida and its Speciation in Various Samples in a Tertiary Care Hospital in North Karnataka, India. Int. J. Curr. Microbiol. App. Sci; 4(9): 996-1000.
26. Sachin CD, Saini S and Mathew S (2014): Non-albicans Candida Infection: An Emerging Threat. Interdisciplinary Perspectives on Infectious Diseases; 1:1-7.
27. Souza MN, Ortiz SO, Mello MM, et al. (2015): Comparison between four usual methods of identification of Candida species. Rev. Inst. Med. Trop. SaoPaulo; 57(4):281-287.
28. Sow DB, Fall M, Ndiaye M, et al., (2015): Usefulness of MALDI-TOF mass spectrometry for routine identification of Candida species in a resource-poor setting. Mycopathologia, 180: 173-179.
29. Ullmann AJ, Akova M, Herbrecht R, et al (2012): ESCMID guideline for the diagnosis and management of Candida diseases 2012: adults with haematological malignancies and after haematopoietic stem cell transplantation (HCT). ClinMicrobiol Infect 18(7): 53-67.
30. Van Veen SQ, Claas EC and Kuijper EJ (2010): High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization time-of-flight mass spectrometry in conventional medical microbiology laboratories. J. Clin. Microbiol, 48: 900-907.
31. Dutta V, Lyngdon WV, Bora I et al. (2015): Characterization of Candida Species from Intensive Care Unit Isolate in a Tertiary Care Center in North East India: A Retrospective study. Int. J. of Medicine and Public Health; 5: 312-316.
32. Yu¨ cesoy M, Esen N, Yulug˘ N. (2001): Use of chromogenic tube and methyl blue-sabouraud agar for the identification of Candida albicans strains. Kobe J Med Sci;47:161-7.
33. Cornet M, Sendid B, Fradin C, et al (2011): Molecular identification of closely related candida species using two ribosomalintergenic spacer fingerprinting methods. J Mol Diagn; (13):12–22.
34. Eraso E, Moragues MD, Villar-Vidal M, et al (2006): Evaluation of the New Chromogenic Medium Candida ID 2 for Isolation and Identification of Candida albicans and Other Medically Important Candida Species, Journal Of Clinical Microbiology, 44 (9): 3340-3345.
35. Letscher-Bru V, Meyer MH, Galoisy AC, et al (2002): Prospective evaluation of the new chromogenic medium Candida ID, in comparison with Candiselect, for isolation of molds and isolation and presumptive identification of yeast species. J. Clin. Microbiol. 40:1508-1510.
36. Miceli MH, Díaz JA and Lee SA (2011): Emerging opportunistic yeast infections, Lancet Infect Dis; 11: 142-51.
37. Cherkaoui A, Hibbs J, Emonet S, et al (2010): Comparison of two matrix-assisted laser desorption ionization time of-flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J ClinMicrobiol;48 (4):1169-75.

2/4/2020