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Characterization and Identification of Atypical Yeast Species Causing Fungemia by MALDI-TOF MS Technique

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ABSTRACT

The incline healthcare services and improvements in technology have elevated resistance or confrontation amongst microorganisms. The microbial community has witnessed evolution by emerging variant yet similar strains which are hard to detect and treat. An upsurge in drug resistance has hit at last; overuse of drugs should be prohibited otherwise the future is about to come when there will be no antibiotic left to control an illness. Prescription of suitable drugs must be mandatory following accurate diagnostics to the disease causative agents or factors. The Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) represents new era of diagnostics and medical microbiology. This study focus on utilization of MALDI-TOF MS technique for characterization and identification of rare yeast species as clinical isolates screened from critically ill patients with fungal infection. The commencement of antifungal resistance in a wide spectrum of atypical yeasts causing fungemia in several hospitals of India is worrisome. The rising of hospital patients with compromised immunity are giving way to pathogenic fungi leading to death of thousands patients. At lower differential neutrophills count, the invasion of uncommon yeast *Candida albicans* (Fungi: Saccharomycetaceae) be able to root a systemic infection lead to surplus growth of pathogenic rare yeasts in many organs, and possibly fatal. At this stage, physicians' often recommend anti-fungal drugs to keep the controlled yeast populace in patients. But over the time, main challenge to medical experts during treatment is the development or emergence of resistance in rare yeast species against the prescribed drugs, insisting medical and pharmaceutical scientists to design and formulate more potent drugs and device rapid, truthful diagnostic techniques.

KEYWORDS: MALDI-TOF MS, Fungemia, Candidemia, Rare yeast, *Candida* species, Rapid diagnosis.

INTRODUCTION

Since ancient period yeasts and fungi have utilized reasonably good associations with humans. These are cosmopolitan and ubiquitous in abundance, and appear on plant leaves, flowers, soil, salt water, baked goods and beer, as well as in animal physiological system including gastrointestinal tracts and skin surfaces, but some verities of yeasts and fungi might be able to set off diseases in healthy people

also (Kohler et al., 2015). Fungemia is the occurrence of fungi or yeasts in the blood stream. The most common type of fungemia is caused by *Candida* species, also known as candidemia, candedemia, or systemic candidiasis; and can be defined as the presence of *Candida* species in the blood. The candidemia is known to among the most widespread bloodstream infections of any kind (Kullberg et al., 2015; Shoff and Perfect, 2020). *C. albicans* is the most abundant strain of candidemia, representing 35% to 60% of isolates responsible for cause of effortlessly curable ailments like vaginitis, diaper rash and oral thrush (Angular et al., 2015; van Schalkwyk and Yudin, 2015). But according to some reports, yeasts and fungi are affecting some population comparatively more severely those who diagnosed with immuno-compromised physiological systems (e.g., cancer, AIDS, diabetes, and organ transplant patients) are succumbing to fungal pathogens, leading to thousands of deaths in a year (Moran et al., 2009; Badiee and Hashemizadeh, 2014). At the lower neutrophill cell count in peoples, the exposure and invasion of uncommon yeast *C. albicans* can lead to a systemic infection and patients may died ultimately due to heavy burden of yeast in various organs. At this stage of systemic infection, the medical consultants and physicians often move to anti-fungal drugs to keep the level of yeast under control (MacCallum, 2009). But over the time these rare yeast strains may evolved with resistance or tolerance to ongoing treatments and drugs, forcing medical practitioners and Pharma researchers to design, formulate more potent drugs as well as device and employed rapid diagnostic techniques for better, swift and truthful treatment (Moyes et al., 2011). The *Candida* sp. infection (candidemia) in immune-sensitive patients is the fourth most common bloodstream infection in hospitals with nearly 40-50% of mortality rate (Li et al., 2016; Rodrigues et al., 2019). The treatment is quite narrower in range to few marginally effective anti-fungal drugs that produce momentous side effects and to which the pathogens adapting increasingly resistant nature (Jia et al., 2018; Dudoignon et al., 2019).

The advancement in medical and surgical management practices of patients over past three decades, yeasts along with some fungi have been emerged out in the form of major cause of human disease. Of these scattered mycoses, candidiasis leftovers the most rampant, with *C. albicans* causing more invasive infections than any other fungus (Tedec et al., 2016). However, the augmenting populace of immune-susceptible patients, especially those having impaired physiological immune system, has led to the appearance of opportunistic infection with less pathogenic atypical yeasts or harmless commensals. The incidence of candidiasis has augmented spectacularly over the past 25-30 years, and similar pattern inevitably continue at present century also. In recent decades just over 40 out of more than 200 known *Candida* species have been caught up in human infections, although only a few cause invasive infection on a regular basis and reported in many studies as epidemiology of candidemia. At large, the defined hierarchy was unswerving from consecutive studies, and the ranking of top five species (*C. albicans, C. glabrata, C. parapsilosis, C. krusei,* and *C. tropicalis*) was also consistent over a 6.5 year period from 1997 to 2003 in a global scenario of invasive candidiasis (White et al., 1998; Sardi et al., 2011). Invasive procedures *vs.* immune-suppressive illness including organ transplantation, chemotherapy, AIDS, diabetes mellitus and over use of extended spectrum antibiotics had contributed to the augmented candid infections (Bassetti et al., 2017). Three classes of antifungal drugs including polyenes, azoles and echinocandins are available for the treatment of Invasive Candidiasis (IC) (Perlin et al., 2007).

Candida species are one of the most common fungal infections in these patients and mainly acquired endogenously as normal human flora. The infections due to *Candida* species encompass a wide range of clinical appearance ranging from superficial to systemic and potentially life-threatening infections. Traditionally, the mycological diagnosis of *Candida* species is based on germ tube formation, colony morphology on rice meal Tween 80 agar and biochemical tests (Joshi et al., 1993; Deorukhkar and Roushani, 2018). Species level identification of yeast can be performed on the basis of assimilation/fermentation test using automated and semi-automated systems (Pincus et al., 2007). *C. krusei* is well known as a fungal pathogen for patients with hematologic malignancies and transplant recipients has been recognized as a potentially multidrug-resistant (MDR) due to its intrinsic fluconazole resistance (Pfaller et al., 2008). Though the fresh raise in abundance these infections is principally coupled to *C. albicans*, non-albicans *Candida* (or NAC: *C. rugosa C. tropicalis, C. parapsilosis, C. guilliermondii*, *C. kefyr C. krusei* and *C. glabrata*) related diseases. *Saccharomyces* are increasingly assessed as agents of invasive infections, especially in immuno compromised or critically ill patients

(Enache-Angoulvant and Hennequin, 2005; Atici et al., 2017). *Candida* and *Aspergillus* species are the most common cause of invasive fungal infection (IFI) in incapacitated individuals' pathogens, causing great morbidity and mortality in those patients (Badiee and Hasehemizadeh, 2014).

The changing in recent years depending on the setting and clinical features, the *Candida* species showed variations in its response to antifungal drugs. Therefore, *Candida* species identification and antifungal susceptibility of testing notified a critical importance for the selection of the appropriate antifungal drugs. It is key interest to analyze the species distribution and susceptibility profile of *Candida* isolates to contribute to the local and nationwide surveillance data in order to assist the treatment planning of patients with *Candida* infections. Diagnosis is an important initial step in treatment of a disease. The poor diagnosis or delayed diagnosis leads to life-threatening results, even to a susceptible strain (Kumar et al., 2018; Updhyay et al., 2019). The early methods of diagnosis integrated conventional methods of biochemical tests followed by molecular identification and advanced technologies, like proteome analysis. The Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) represents the new era of diagnostics and microbiology. The advancement in sample processing and expansion of databases has enhanced diagnosis of microorganisms from small clinical samples (Sanchez et al., 2019). During the yester decade, applicability of MALDI-TOF MS has made easy and rapid diagnosis of pathogen directly from clinical samples, eliminating the need for culturing. Moreover, MALDI-TOF MS has also contributed to antimicrobial susceptibility testing (AST) by producing reliable same-day results and by passed the 24hrs incubation of routinely performed AST (Welker and van Belkum, 2019). Detection of early nosocomial outbreaks using MALDI- TOF MS by bacterial typing could help in putative beneficial impact on control of the disease along with patient safety (Clerc et al., 2012; 2013). In the present study, preliminary techniques were used initially along with the confirmation of *Candida* species using MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) technology after Wolters et al. (2011) and Freitas et al. (2017). There is no clinical approach to the study established in this document. So that the present study aimed to find the correct, truthful and rapid diagnostic tool of pathogenic rare yeast species and to assess their antifungal susceptibility pattern, which might be helpful in disease diagnosis, management and swift treatment.

FUNGEMIA

Fungemia is an emerging clinical entity worldwide due to rare yeast infections (Michael et al., 2005; Dasguptaet al., 2015). Fungemia has been historically considered to be a disease caused by a single *Candida* species, to be called Fungal Blood stream Infection (FBSI). The fungemia has been classically considered to be a monomicrobial disease and the detection of atypical or rare species of yeast in circulating blood was distinctly uncommon using traditional microbiological procedures (Pappas et al., 2003; Boktour et al., 2004; Jensen et al., 2007). It is important to accurately identify these species of rare yeasts which are intrinsically resistant to certain classes of antifungal agents (Ahmad et al., 2012). The term candidemia describes the occurrence of *Candida* species in the blood is a most common manifestation of invasive candidiasis. *Candida* in a blood culture should never be viewed as a contaminant and should always prompt a search for the source of the bloodstream infection. For many patients, candidemia is a manifestation of invasive candidiasis that could have originated in a variety of organs (Fridkin, 2005). *Candida* yeasts are detectable in 96% of neonates by the end of the first month of life. The special anatomical and physiological features of the individual compartments of the mouth, stomach and intestine offer disparate ecological niches and they are colonized with site-specific microbe communities (Schulze and Sonnenborn, 2009).

Candida krusei was firstly identified in 1839 by Langenbeck from a typhus patient, however after 75 years of this Castellani suggested its disease causing activity in humans. The *C. krusei* is generally considered to be a momentary commensal and occasionally isolated from mucosal surfaces but since middle of 20th century an increase in reports of *C. krusei* as a human pathogen was recorded (Samaranayake and Samaranayake, 1994). On contrary to ovoid candids, *C. krusei* are generally elongated similar to *C. kefyr* (formerly known as *C. pseudotropicalis*). The *C. krusei* has a variety of colony topologies. The ultra topology of multilayered cell wall showed outer most irregular coat of

flocculent material, an electron-dense zone, a granular layer, a less granular layer, a thin layer of dense granules and another sparsely granular layer outside the cell membrane. This superb architecture variations account for the differential behavior of *C. krusei* in biological fluids such as saliva and bronchial lavage fluid comparing with other *Candida* species (Coleman et al., 1997; Gutierrez et al., 2002; Bassetti et al., 2006). The *C. krusei* has two basic morphological forms i.e. yeast and pseudohyphae; and both are often present concurrently and could not separate simply. *C. krusei* grows at a 37°C but can withstand temperature up to 45°C in a vitamin-free media (Scorzoni et al., 2013). Similar to *C. auris, C. krusei* can adhere to abiotic surfaces but not to as *C. albicans*, which is essential for colonization and invasion. The *C. krusei* produce most extensive biofilm on the surfaces of polyvinyl chloride catheter disks regardless of the growth medium similar to more pathogenic *C. albicans* on contrary to less pathogenic species, *C. parapsilosis, C. pseudotropicalis* (now *C. kefyr*) and *C. glabrata* (Jensen et al., 2011). *C. krusei* does not adhere to buccal epithelial cells whereas *C. tropicalis, C. parapsilosis, C. guilliermondii*, and *C. kefyr* do so. The susceptibility to lysozyme, an antimicrobial enzyme produced in phagosomes has been used as a method to assess the microbial virulence that indicated pattern of susceptibility to lysozyme as: *C. krusei* > *C. parapsilosis* > *C. tropicalis* > *C. guilliermondii* > *C. albicans* > *C. glabrata*, *i.e.* the latter being the most resistant to lysozyme (Neppelenbroek et al., 2013). The pathogen profile can be stated as: diploid, no relation to CTG clade, genome sequence available, high antifungal resistance and minimal molecular laboratory tools availability. *C. kefyr* was at first isolated recovered from dairy products and named *Saccharomyces fragilis*, latter on called *C. pseudotropicalis*, and reclassified as *Kluyveromyces marxianus*. It is rarely associated to disease contributing about 1% isolates of *Candida* species from clinical specimens. The first report of invasive *C. kefyr* (*C. pseudotropicalis*) was identified in a patient with metastatic adenocarcinoma of breast. It can colonize to oral cavities, gastrointestinal tract, and urinary tract of immune-compromised patients with several potential risk factors (Morgan et al., 1984). It is generally thought that their conversion from commensalism to parasitism is largely determined by the host immune (Dufresne et al., 2014). The pathogen profile can be stated as: ploidy is not determined, does not belong to CTG clade, Genome sequence available, antifungal resistance is moderate, few molecular laboratory tools available.

C. rugosa rarely causes invasive infections; however, recently, isolates have been shown to be an increasing cause of fungal infections especially in Latin America. Besides, *C. rugosa* appears decreased susceptibility to fluconazole with various patterns following geographic regions (Pfaller et al., 2008). The pathogen profile can be stated as: haploid, belongs to CTG clade, genome sequence is not available, antifungal resistance is high, few molecular laboratory tools available. The epidemiology of invasive fungal infections (IFIs) has been difficult to clean from the literature because of the different definitions used, the different risk groups studied, and variation from institution to institution. Approximately 80% of IFIs are due to *Candida* species. Invasive candidiasis comprises both candidemia and deep-seated tissue candidiasis (Handal et al., 2015). The other rare yeast and filamentous fungi belonging to hyalohyphomycetes (*Scedosporium, Fusarium*), zygomycetes and dematiaceous (*Alternaria, Bipolaris*), and *Trichosporon* are implicated in human infections ranging from colonization to localized. Amphotericin B is the drug of choice for most rare yeast infections except for *Trichosporon* (Wisplinghoff et al., 2004; Pappas et al., 2006; Pfaller et al., 2007; Sievert et al., 2013; Nur et al., 2014).

MALDI-TOF MS

Within a decade, Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) has become a gold standard in species level identification of microorganisms (Schubert and Kostrzewa, 2017). MALDI-TOF mass spectrometry is an analytical technique which involves ionization of chemical compounds into charged molecules followed by detection based on mass/charge (m/z) ratio measurement. In the present study, the microorganism of choice is *Candida,* a thrush pathogen belonging to rare yeast species for candidiasis. The high demand of MALDI-TOF MS in clinical laboratories is due to easy, rapid, cost-effective and reliable diagnosis of microorganisms. A fresh 24hrs culture is used to identify microorganisms at very high accuracy using direct and indirect identification methods. Direct identification involves direct loading of the culture

over the wells on the metal target plate, followed by a drop of 70% formic acid covering the dried sample and coated the dried well with a suitable matrix solution, whereas processing is required for certain cultures for extraction of internal proteins, in indirect approach (Stevenson et al., 2010). For rare yeasts, ethanol/formic acid extraction method is devised to give promising results. A suitable matrix used in MALDI-TOF MS is α-Cyano-4-hydroxycinnamic acid. After processing the candids sample to extract internal ribosomal proteins, matrix is overlaid on dried sample well prior to subjecting it to the mass spectrometer.

Proteins are fragile biomolecules and get easily fragmented on administration to ionizing radiation, hence the matrix provides protection to the underlying protein by absorbing the radiation directly by using short pulse of laser instead of continuous exposure. MALDI Biotyper 3.1 Microflex (Bruker Daltonics, GmbH, Bremen, Germany) uses ultraviolet (UV) laser for the purpose of ionization. The laser irradiates the matrix-sample mixture, evaporating the matrix and releasing positively charged proteins in a so-called 'soft' ionization process (Clark et al., 2013). The ability of the matrix to absorb UV light and transfer of protons onto the extracted proteins is crucial to this process. The protein ions are electrostatically accelerated over a short distance and arrive in flight tube at a speed that is proportional to their mass. Protein ions with different masses arrive at the detector after different time periods. Simply by measuring time between pulsed acceleration and the corresponding detector signal (in nano-second range), the speed of the ions can be measured very precisely and converted into an accurate molecular mass.

Identification of microorganisms is performed by comparison of generated Peptide Mass Fingerprint (PMF) with the reference PMFs in the database. Once a match is found, the score value is generated. The results are displayed with values >1.7 corresponding to genus similarity; and values >2.0 confirming species-level identification. MALDI Biotyper 3.1 Microflex (Bruker) uses Flex control software for Real time Classification (RTC) of the microorganisms Basic and most essential step of the health care industry belongs to timely and accurate identification of the causative agent of a disease. MALDI-TOF MS is evolving in phases to become the leading technology in investigating the pathogen. MALDI-TOF MS has been used to provide images of the proteins identified in the cell which shows the abundance of the respective proteins in the organism (Dunham et al., 2016).

MALDI-TOF MS *VS.* CONVENTIONAL METHODS

In recent scenario, identification of organisms is best conducted using 16S rRNA and 18S rRNA in gene sequencing. However, the emergence of MALDI-TOF MS has revolutionized the methods of identification in clinical microbiology. MALDI- TOF MS produces species-specific protein fingerprints. This mass spectrum holds molecular masses of nearly all ribosomal and few other proteins. Such data is compared with a reference database to allow comparative identification of a microorganism (Singhal et al., 2015). The conventional methods including Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), 2-Dimensional Gel Electrophoresis (2-DGE) and molecular techniques have been employed in the past for better identification of microorganisms. The SDS-PAGE proteome separation coupled with computer-based analysis had been used but was not appreciated much among microbiologists. This could have occurred due to requirement of standardized environmental conditions, use of toxic chemicals, and lack of reference proteome for certain microorganisms and its incapability to differentiate highly similar strains. The labor intensive 2-DGE also failed to satisfy the requirement of the microbiologists (Saeed et al., 2017; Fallahi et al., 2020).

CONCLUSIONS

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) can speedily exemplify microorganisms by generating mass spectra of proteins that are compared to known databases. If those proteins could be identified as well, the pathogenesis of the bacteria and the mechanisms of resistance could be understood too. Moreover the detection of toxins has been achieved in some cases using MALDI-TOF MS. MALDI-TOF MS has a mandatory requirement of a 'reference database' which includes peptide mass fingerprint (PMF) of internal ribosomal proteins of

different organisms. Various studies have described the importance and involvement of MALDI-TOF MS for species characterization. Prescription of appropriate drugs must be mandatory following an accurate diagnostic of the causative agent. In-spite of MALDI-TOF MS being a breakthrough, the associated drawbacks is a major concern. Correction of the limitations will lead to a potential diagnostic system with very high reliability of results

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REFERENCES

1. Aguilar G., Delgado C., Corrales I., Izquierdo A., Gracia E., Moreno T., Romero E., Ferrando C., Carbonell J.A., Borrás R., Navarro D. and Belda F.J. (2015). Epidemiology of invasive candidiasis in a surgical intensive care unit: An observational study. *BMC Res. Notes*., 29(8): 491.
2. Ahmad, S. and Khan, Z. (2012). Invasive candidiasis: A review of nonculture-based laboratory diagnostic methods. *Indian J. Med. Microbiol.,* 30: 264–269.
3. Atici S., Soyal A., Cerit K.K., Yilmaz S., Aksu B., Kiyan G. and Bakir M. (2017). Catheter-related *Saccharomyces cerevisiae* fungemia following *Saccharomyces boulardii* probiotic treatment: In a child in intensive care unit and review of the literature. *Med. Mycol. Case Rep*., 15: 33–35.
4. Badiee P. and Hasehemizadeh Z. (2014). Opportunistic invasive fungal infections: Diagnosis and clinical management. *Indian J. Med. Res*., 139(2): 195–204.
5. Bassetti M., Poulakou G., Ruppe E., Bouza E., Van Hal S.J. and Brink A. (2017). Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: A visionary approach. *Intensive Care Med.*, 43(10): 1464–1475.
6. Bassetti M., Righi E., Costa A., Fasce R., Molinari M.P., Rosso R. and Viscoli C. (2006). Epidemiological trends in nosocomial candidemia in intensive care. *BMC Infect. Dis.,* 6(1): doi:10.1186/1471-2334-6-21
7. Boktour M., Kontoyiannis D.P., Hanna H.A., et al. (2004). Multiple-species candidemia in patients with cancer. *Cancer,* 101: 1860–1965.
8. Clark A.E., Kaleta E.J., Arora A. and Wolk D.M. (2013). Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry: A fundamental shift in the routine practice of clinical microbiology. *Clin. Microbiol. Rev.,* 26(3): 547–603.
9. Clerc O., Prodhom G., Vogne C., Bizzini A., Calandra T. and Greub G. (2012). Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the clinical management of patients with gram-negative bacteremia: A prospective observational study. *Clin. Infect. Dis.,* 56(8): 1101–1107.
10. Coleman D.C., Sullivan D.J., Bennett D.E., Moran G.P., Barry H.J. and Shanley D.B. (1997). Candidiasis: The emergence of a novel species, *Candida dubliniensis*. *AIDS*, 11: 557–567.
11. Dasgupta S., Das S., Chawan N.S. and Hazra A. (2015). Nosocomial Infections in the Intensive Care Unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India. *Indian J. Crit. Care Med.*, 19(1): 14–20.
12. Deorukhkar S.C. and Roushani S. (2018). Identification of *Candida* species: Conventional methods in the era of molecular diagnosis. *Ann. Microbiol. Immunol*., 1(1): 1–6.
13. Dudoignon E., Alanio A., Anstey J., Depret F., Coutrot M., Fratani A., Jully M., Cupaciu A., Chaussard M., Oueslati H., Ferry A., Benyamina M., de Tymowski C., Boccara D., Serror K., Chaouat M., Mimoun M., Lafaurie M., Denis B., Gits-Muselli M., Bretagne S., Mebazaa A., Legrand M. and Soussi S. (2019). Outcome and potentially modifiable risk factors for candidemia in critically ill burns patients: A matched cohort study. *Mycoses*., 62(3): 237–246.
14. Dufresne S.F., Marr K.A., Sydnor E., Staab J.F., Karp J.E., Lu K., Zhang S.X., Lavallee C., Perl T.M. and Neofytos D. (2014). Epidemiology of *Candida kefyr* in patients with hematologic malignancies. *J. Clin. Microbiol.*, 52(6): 1830–1837.
15. Dunham S.J., Ellis J.F., Li B. and Sweedler J.V. (2016). Mass spectrometry imaging of complex microbial communities. *Acc. Chem. Res.,* 50(1): 96–104.

16. Enache-Angoulvant A. and Hennequin C. (2005). Invasive *Saccharomyces* infection: A comprehensive review. *Clin. Infect. Dis*., 41(11): 1559–1568.
17. Fallahi S., Babaei M., Rostami A., Mirahmadi H., Arab-Mazar Z. and Sepahvand A. (2020). Diagnosis of *Candida albicans*: Conventional diagnostic methods compared to the Loop-Mediated Isothermal Amplification (LAMP) assay. *Arch. Microbiol*., 202(2): 275–282.
18. Freitas A.R., Sousa C., Novais C., Silva L., Ramos H. and Coque T.M. (2017). Rapid detection of high-risk *Enterococcus faecium* clones by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Diagn Microbiol Infect. Dis.,* 87(4):299–307.
19. Fridkin S.K. (2005). The changing face of fungal infections in health care settings. *Clin. Infect. Dis.,* 41: 1455.
20. Gutiérrez J., Morales P., González M.A. and Quindós G. (2002). *Candida dubliniensis*: a new fungal pathogen. *J. Basic Microbiol*., 42(3): 207.
21. Handal N., Jorgensen S.B., Tunsjø H.S., Johnsen B.O. and Leegaard T.M. (2015). Anaerobic blood culture isolates in a Norwegian university hospital: Identification by MALDI-TOF MS *vs* 16S rRNA sequencing and antimicrobial susceptibility profiles. *Apmis.,* 123(9): 749–758.
22. Jensen J., Munoj P., Guinea J., Rodriguez-Creixems M. Pelaez T. and Bouza E. (2007). Mixed fungemia: Incidence, risk factors, and mortality in a general hospital. *Clin. Infect. Dis.,* 44(12): e109–e114.
23. Jensen K., Santisteban M.S., Urekar C and Smith M.M. (2011). Histone $H2_{A.Z}$ acid patch residues required for deposition and function. *Mol. Genet. Genomics.,* 285(4): 287–96.
24. Jia X., Li C., Cao J., Wu X. and Zhang L. (2018). Clinical characteristics and predictors of mortality in patients with candidemia: A six-year retrospective study. *Eur. J. Clin. Microbiol. Infect. Dis*., 37(9): 1717–1724.
25. Joshi K.R., Solanki A. and Praksh P. (1993). Morphological identification of *Candida* species on glucose agar, rice extract agar and corn meal agar with and without Tween-80. *Indian J. Pathol. Microbiol*., 36(1): 48–52.
26. Kohler J.R., Casadevall A. and Perfect J. (2015). The spectrum of fungi that infects humans. *Cold Spring Harb. Perspect. Med.*, 5(1): a019273.
27. Kullberg B.J. and Arendrup M.C. (2015). Invasive candidiasis. *N. Engl. J. Med.*, 373(15): 1445–1456.
28. Kumar P., Upadhyay S.K. and Singh R. (2018). A study on recent trends in therapy of air borne communicable disease caused by *Mycobacterium tuberculosis*: The Tuberculosis. *Bull. Pure Appl. Sci. (Zool),* 37A(2): 65–74.
29. Li Y., Du M., Chen L.A., Liu Y., Liang Z. (2016). Nosocomial bloodstream infection due to *Candida* spp. in China: Species distribution, clinical features, and outcomes. *Mycopathol.,* 181(7-8): 485–495.
30. MacCallum D.M. (2009). Massive induction of innate immune response to *Candida albicans* in the kidney in a murine intravenous challenge model. *FEMS Yeast Res*., 9(7): 1111–1122.
31. Michael Y.L., Yehuda C., Jennifer Z., Flores E.L., Tolentino J., Pranavi S. and Weber S.G. (2005). Prior antimicrobial therapy and risk for hospital-acquired *Candida glabrata* and *Candida krusei* fungemia: A case-case-control study. *Antimicrob. Agents Chemother.,* 49(11): 4555–4560.
32. Moran C., Grussemeyer C.A., Spalding J.R., Benjamin D.K. and Reed S.D. (2009). *Candida albicans* and non-albicans bloodstream infections in adult and pediatric patients: Comparison of mortality and costs. *Pediatr. Infect. Dis. J.,* 28(5): 433–435.
33. Morgan M.A., Wilkowske C.J. and Roberts G.D. (1984). *Candida pseudotropicalis* fungemia and invasive disease in an immunocompromised patient. *J. Clin. Microbiol*., 20(5): 1006–1007.
34. Moyes D.L., Murciano C, Runglall M., Islam A., Thavaraj S., and Naglik J.R. (2011). *Candida albicans* yeast and hyphae are discriminated by MAPK signaling in vaginal epithelial cells. *PLoS One*, 6: e26580.
35. Neppelenbroek K.H., Seo R.S., UrbanV.M., Silva S., Dovigo L.N., Jorge J.H. and Campanha N.H. (2014). Identification of *Candida* species in the clinical laboratory: A review of conventional, commercial, and molecular techniques. *Oral Dis.,* 20(4): 329–44.
36. Nur Y. (2014). Epidemiology and risk factors for invasive candidiasis, *Ther. Clin. Risk Manag.,* 10: 95–105.
37. Pappas P.G. (2006). Invasive candidiasis. *Infect. Dis. Clin. North Am.*, 20(3): 485–506.

38. Pappas P.G., Rex J.H. and Lee J. (2003). A prospective observational study of candidemia: Epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. *Clin. Infect. Dis.,* 37: 634–643.
39. Perlin D.S. (2007). Resistance to echinocandin-class antifungal drugs. *Drug Resist. Updates*, 10(3): 121-130.
40. Pfaller M.A. and Diekema D.J. (2007). Epidemiology of invasive candidiasis: A persistent problem. *Clin. Microbiol. Rev.,* 20(1): 133–163.
41. Pfaller M.A., Diekema D.J., Gibbs D.L., Newell V.A., Nagy E., Dobiasova S., Rinaldi M., Barton R. and Veselov A. (2008). *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: Geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. *J. Clin. Microbiol.,* 46(2): 515–521.
42. Pincus D.H., Orenga S. and Chatellier S. (2007). Yeast identification: Past, present, and future methods. *Med. Mycol.*, 45(2): 97–121.
43. Rodrigues C.F., Rodrigues M.E. and Henriques M. (2019). *Candida* sp. infections in patients with diabetes mellitus. *J. Clin. Med.*, 8(1): 76.
44. Saeed S., Hasan S., Kuldeep and Parmar S.S. (2017). Conventional and recent diagnosis aids in oral candidal infections: A brief overview. *Biomed. Pharmacol. J.*, 10(1): 419–426.
45. Samaranayake Y.H. and Samaranayake L.P. (1994). *Candida krusei*: Biology, epidemiology, pathogenicity and clinical manifestations of an emerging pathogen. *J. Med. Microbiol.*, 41(5): 295–310.
46. Sanchez B.R., Cercenado E., Coste A.T. and Greub G. (2019). Review of the impact of MALDI-TOF MS in public health and hospital hygiene, 2018. *Euro. Surveill.,* 24(4): 1800193.
47. Sardi J.C., Almeida A.M. and Mendes-Giannini, M.J. (2011). New antimicrobial therapies used against fungi present in subgingival sites: A brief review. *Arch. Oral. Biol*., 56: 951–959.
48. Schubert S. and Kostrzewa M. (2017). MALDI-TOF MS in the microbiology laboratory: Current trends. *Curr. Issues Mol. Biol.,* 23: 17–20.
49. Schulze J. and Sonnenborn U. (2009). Yeasts in the Gut: From commensals to infectious agents. *Dstch. Arztebl. Int.,* 106(51-52): 837–842.
50. Scorzoni L., de Lukas M.P., Mesa-Arango A.C., Fusco-Almeida A.M., Lozano E., Cuenca-Estrella M., Mendes-Giannini M.J. and Zaragoza O. (2013). Antifungal efficacy during *Candida krusei* infection in non-conventional models correlates with the yeast *in vitro* susceptibility profile. *PLoS One,* 8(3): e60047.
51. Shoff C.J. and Perfect J.R. (2020). Uncommon yeasts and molds causing human disease. *Ref. Module Life Sci.,* Doi.org/10.1016/B978-0-120809633-8.21023-6.
52. Sievert D.M., Ricks P. and Edwards J.R (2013). National Healthcare Safety Network (NHSN) team and participating NHSN Facilities antimicrobial-resistant pathogens associated with healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention 2009–2010. *Infect Cont. Hosp. Epidemiol.,* 34(1): 1–14.
53. Singhal N., Kumar M., Kanaujia P.K. and Virdi J.S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front. Microbiol.,* 6: 791.
54. Stevenson L.G., Drake S.K. and Murray P.R. (2010). Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin. Microbiol.*, 48(2): 444–447.
55. Tadec L., Talarmin J.P., Gastinne T., Bretonnière C., Miegeville M., Le Pape P., Morio F. (2016). Epidemiology, risk factor, species distribution, antifungal resistance and outcome of Candidemia at a single French hospital: A 7-year study. *Mycoses.* 59(5): 296–303.
56. Upadhyay S.K., Singh R., Kumar P., Singh M., Yadav M., Kumar V., Aggarwal D. and Sehrawat N. (2019). *In vitro* antitubercular activity of dihydropyridine-dicarboxamide and pyrazole derivatives against *Mycobacterium tuberculosis*. *Bull. Pure Appl. Sci. (Zool.),* 38A(2): 102–109.
57. van Schalkwyk J. and Yudin M.H. (2015). Vulvovaginitis: Screening for and management of trichomoniasis, vulvovaginl candiasis and bacterial vaginosis. *J. Obstet. Gynaecol. Can.,* 37(3): 266–274.
58. Welker M. and van Belkum A. (2019) one system for all: is mass spectrometry a future alternative for conventional antibiotic susceptibility testing? *Front Microbiol.,* 10: 2711.

59. White J.M., Chaudhry S.I., Kudler J.J., Sekandari N., Schoelch M.L. and Silverman S.Jr. (1998). Nd:YAG and CO2 laser therapy of oral mucosal lesions. *J. Clin. Laser Med. Surg.,* 16: 299–304.
60. Wisplinghoff H., Bischoff T., Tallent S.M., Seifert H., Wenzel R.P. and Edmond M.B. (2004). Nosocomial bloodstream infections in US Hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin. Inf. Dis., 39*(3): 309–317.
61. Wolters M., Rohde H., Maier T., Belmar-Campos C., Franke G., Scherpe S. and Christner M. (2011). MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant *Staphylococcus aureus* lineages. *Int. J. Med. Microbiol.,* 301(1): 64–68.