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Diagnosis of Invasive Candidiasis: A Narrative Review

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Abstract

In recent years, the occurrence of opportunistic fungal infection effect in the hospital environment. Candida and Aspergillus are main cause of fungal infections. Candida infection is widespread in Blood Stream Infections (B.S.I.). Candidemia is a common infection with high morbidity and mortality rate og infection in patients with haematological malignancies who are seriously ill. Bloodstream infection are caused due to prolonged stay in hospitals, use of broad-spectrum antibiotics and during or after chemotherapy. Because of the rise in antimicrobial resistance and a small supply of antifungal medication. Immune-compromised patients have a high chance of infection due to several comorbidities and the lack of specificity in blood culture based approaches with the detection of particular species leading to misdiagnosis within strictly associated and time consuming species. The main focus of this review is on the epidemiology, pathogenesis, diagnosis, clinical manifestations, and management of Invasive Candidiasis. Differentiation of Candidemia from bacterial infection is difficult and complex process. Five species result from over 90% of invasive infections with Candida species: Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis and Candida krusei. However, several species of Candida have been successfully isolated and identified from clinical samples. The paucity of new techniques for the rapid detection to overcome with the time consuming traditional methods. In this study we summarized the recent advancement in microbiology for the detection and identification of specific microbial species. Furthermore these findings are helpful for the proper treatment and management of Candidemia infection by the rapid diagnosis and detection.

Keywords: Candida; Immuno-compromised; Diagnosis; Blood Stream Infection

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Introduction

Invasive Candidemia or Candidiasis (IC) infection is one of the prominant and also is painful fungal infection to treat in clinical practice, due to the increase usage of potent and novel immunosuppressive agents or organ transplant recipient and chemotherapy advances for haematological malignancies and neutropenic patients. The biggest group at risk for Candidemia and Candida species infections is those who were admitted to the intensive care unit (I.C.U.) [1]. Invasive Fungal disease is an infection that can be caused by various species of yeast (mostly Candida species) as they are opportunistic organism and ubiquitously present in nature [2-5]. The emergence of fungemia especially Candidemia increase steadily, especially in hospitalized patients. Fungal infection/ Invasive fungal Disease (I.F.D.), now a day's become a severe health problem mostly in the people with immune system impairment and also it become the reasonable cause of worldwide mortality [3]. During the past three decades, the number of cancer patients with I.F.D. has rapidly emerged as a global threat to the health of the population in the natural ecosystem [6,7]. Patient with different type of leukemia and bone marrow transplant recipient are more prone to the fungal infections, and health ratios are also increasing from 5% to 40% in the case of acute leukemia alone [7,8]. Each year over 1.6 million deaths occur due to Invasive fungal infections [9]. Prevalence of invasive candidiasis predominates 2.1 to 6.7 per 1000 admissions were found in intensive care units (I.C.U.) [10]. High risk of Candidemia effects premature, low birth weight neonates and occurrence of the highest incidence of Candidemia in neonatal I.C.U. [11]. A major concern for microbiologists and Clinicians around the world is early and timely diagnosis, effective care and prevention of candidiasis. Moreover, among the Candida species, there is an emerging pattern of drug resistance.

Candida is the most common pathogenic genera of the group fungi in which many species like Candida tropicalis, Candida parasilopsis, Candida krusei, Candida formata, Candida lusitanial have been identified. However, Candida albicans being the most common cause of I.F.D., easily and frequently isolated from the human sample [3]. In present years particularly non-albicans. Candidaspecies constitute more massive proportions of candidemia isolates [12]. Prevalence of pathogenic Candida

species distribution depends on the variability in geographic areas; candidiasis is caused by different Candida species which has presented its characteristics like propensity to cause invasive disease, tissue tropism, virulence and antifungal susceptibility while awaiting culture and susceptibility data this information regarding local epidemiology and rate of antifungal resistance is essential to make clinical and therapeutic decisions [11]. In 2009 multidrug-resistant species of Candida auris emerged, this species eventually identified by rDNA sequencing [13]. In India, since 2013, Candida auris has become most important nosocomial pathogen [13]. I.F.D. is an emerging cause of severe public health problem in which 0.20-0.38/1000 admissions are reported from Candidemia alone, being alone the clinically relevant pathogen of the Candida genus [14]. Candida albicans cause 50% of bloodstream infection in United states, Candida glabrata is another most prevelent contributing 25% to 30% in bloodstream infection in patients having age higher than 60 years and in solid organ transplants [11].

Pathogenicity

Virulence factors include hemolytic activity, biofilm formation, proteinase activity, phospholipase activity and esterase activity [15]. Ability to produce (Dimorphism) hyphae and pseudo hyphae (filaments forms) or blastopores (yeast cells) are found at infected site [8]. Ability to cause disease (Virulence) is depending on factors virulence trait & survival traits [8]. Secretion of proteases, hydrolases, adhesion to host tissue, dimorphism and phenotypic switching (reversible alteration of yeast cell morphology) [16]. Favourable host environment conditions are required for the formation of filament is growing at 37◦C like neutral pH and serum [8]. Germ tubes (newly formed filaments) are less adherent to yeast cells than mammalian cells [8,16]. Yeast cells lyse the macrophages and produce filaments to enhance the evasion of host defence mechanism [8]. In the Metschnikowiaceae clade, Candida auris is closely linked to Candida haemulonii phylogenetically [17]. Pathogenicity and virulence factors related to infections with Candida auris are poorly described, so Candida auris can adapt genomically to different environments and has several virulence mechanisms common to Candida albicans and other pathogenic species of Candida [17]. Sometimes, emerging species are not identified by conventional methods, but correct identification was made by correct by MALDI-TOF MS. This system identifies the isolates at the species level with 98.3% accuracy, whereas 96.5% for conventional methods [18].

Candida is unicellular pathogenic species and frequent colonizer of human skin and elementary tract, which cause infection, especially in the immunocompromised population [19]. Bloodstream infections(B.S.I.) are mostly caused by the species of Candida, which is almost 96% of all opportunistic mycosis [20,21]. Candida is actually the fourth most common nosocomial bloodstream in the U.S.A and infections (B.S.I.s) [21,22] and the 5th to 10th most common cause in Europe and Australia [22]. Molecular characterization showing the clonal nature of Candida auris in New Delhi and other regions of India, genotypically distinct from the South Korea and Japanese isolates, revealed the endemic presence of Candida auris [13]. The 6th most common pathogen causing candidemia was Candida auris [4]. The Candida genus becomes pathogenic when it comprises opportunistic fungi in immunocompromised patients [3]. These are polymorphic fungus and got a major portion of the human micro biome [23]. Candida albicans can be found lifelong under a few circumstances, but it can cause severe infections from superficial to the fatal one [23]. Yeast infection spread drastically in immunocompromised patients, I.C.U.s due prolonged stay in hospitals, use of broad-spectrum antibiotics fail to response, febrile neutropenia, and paucity of antifungals [24]. However, the molecular mechanism behind the infection and pathogenicity of Candida species is still complicated [3].

Phylogenetically Candida auris is strongly correlated with Candida haemulonii and in Laboratories Vitek 2 API Auxacolor commercial system misidentified as Candida famata, Candida haemulonii or Rhodotorula glutinis [25]. Candida auris bloodstream infection has the highest rate of mortality ranging from 30-60% [4].

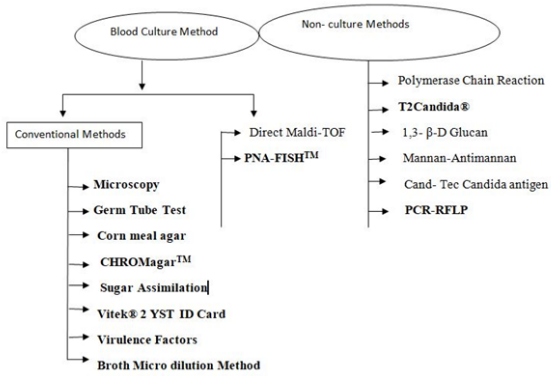

Figure 1 **Algorithm for Diagnosis of Candida Infection**
Systematic representation of conventional and molecular association for diagnosis of Blood stream infection for patients suspected with Candidemia.

Conventional Method

Methods for Candida culture

One of the standard strategies for fungal identification is blood culture. A major concern for Microbiologists and clinicians around the world is early and timely diagnosis, effective care and prevention of candidiasis. Additionally, among the Candida species, there is an emerging pattern of drug resistance. Bactec Plus Aerobic, Bactec Plus Anaerobic and BD Blood culture bottles of mycosis IC/F was used for detection of blood infection [26]. The most widely used blood culture medium is BacT/ALERT® to detect Candida. BacT/ALERT® produces a color shift with pH changes that raise the CO2 concentration using a liquid emulsion sensor and colormetric analysis [27]. Other types of blood culture for Candida detection are Mycosis IC/F blood culture bottles that are extremely specific for Candida species detection [28]. In Bactec 9240 samples incubate until positivity (5 days for aerobic & anaerobic &14 days for mycosis) [28]. BD BackTech TM FX Blood Culture (BC) and VersaTREKTM Method are other forms of blood cultures widely used for diagnosing Candida (AerobicRedox Media) [29]. Combined with full fluorescence sensors, it is a fully automated agricultural machine which provides highthroughput

model analysis.Usually, the VersaTrekTM is a commonly used aerobic stir bar bottle with a magnetic strip. By stirring, the oxygen is invariably mixed with continuous, medium around the media, yielding a stable fungal growth in the bottle [29]. For detection of Candidemia, gold standard blood culture is used traditionally, but this method takes a longer time, and it shows low sensitivity [1]. Positive as well as negative samples were stained with gram stain and cultured on MacConkey and Columbia agar [26]. If the fungus vial test is positive, then gram staining is performed for the confirmation. Alternatively, blood can be collected on plates to form specific colonies. Combinedly, the blood culture and CHROM agar is the gold standard technique for colonization and identification of different Candida Sub-types. Candidaisolates were sub-cultured on Sabouraud dextrose broth (S.D.B.) or Sabouraud dextrose agar (S.D.A.) (Merck Co., Darmstadt, Germany) for 48h at 35◦C [30]. Direct segregation led to the rapid growth of colonies in Charmanagar and, as a result, Candida was rapidly analyzed, although no significant changes in color, shape, and size of the colony were observed (Fig. 4). For differentiation of different Candida species possess different biochemical patterns due to assimilation of carbohydrate and fermentation tests which in turn had to reduce the time [1]. Blood culture is a rather laborious process, despite Substantial advancement in automation and high throughput analysis, and thus not ideal for the rapid diagnosis of Candida. Candida albicans and Candida dubliniensis develops germ tube (GT) that can be used as a morphological attribute distinguish the two species of Candidaquickly. For identification of Candida seeing Colony morphology, Germ tube test (GTT), Candida Chrome Agar (Chromogenic media), Sugar fermentation, sugar assimilation and antifungal susceptibility are done [5]. A new chromogenic medium, CHROM-Pal-Medium (CH-P), Which is an improved version of CHROM agartype, addition with Pal-Agar, has been proposed. Albicans are capable of detecting more than 95%. Therefore some time these tests are not specific to give an accurate result, it delayed the antifungal therapy also [31]. Candidemia causes a high rate of death due to delay in speciation of Candida species by a conventional method; some Candida species are resistant to antifungal drugs [32].98.8 % Candida albicans and 100% Candida krusei were classified by CHROMagar in second survey, 7% Candida tropicalis was described correctly. Candida glabrata was also identified with 98 % sensitivity and 92.1% specificity but some other Candida stains (three stains of Candida species, two stains of Candida lusitaniae, and five out of nine stains of Candida cerevisiae also turned pink. However, the pink color of this strain does not confirmed with Candida glabrata, but it is more likely to go wrong without experienced laboratory personnel. Sivakumar et al , shows a result on CHROM agar of three of the eight Candida tropicalis. A new proposal to use CMA (color, morphology) at the same time used to resolve these shortcomings, and Candida tropicalis with>>99% confidence was found. The most common approachof using carbohydrate identity and fermentation reactions to recognize Candida in a mycology Laboratory traditionally, maltose, glucose, sucrose, lactose rhamnose, and trehalose arebonded with liquid media in test tubes, while glucose, maltose, sucrose and lactose are used for fermentation [33]. Automated biochemicaland assimilation tests have been developed for reducing time like VITEK 2 YST ID and API Candida system (bioMerieux) [1]. Specific treatment with required antifungal agents plays an essential role in reducing the mortality due to candidemia [1]. One of the first commercially available systems to detect Candida is the API 20C AUX system. It is quicker than the conventional methods of blending and fermentation, but involves incubation for 72 hour. Auxacolor and API Candida API 20C is an enhanced version of API 20C AUX and can generate results after 24 hours' time of incubation. Both systems are incorporated to recognize the most predominant clinical pathogens.26 species can be identified by the Auxacolor system, while only 15 species can be identified by the API Candida system. For more popular species, these techniques used the deliver the best identification results with an confirmationrate of 78.8% to 91.8% for API Candida, 85.7% to 91.2% for Auxacolor, 62.1% to 99.3% for API 20C, 89.7% for Vitek Yeast Biochemical Card (YBC), 99.8% for Uni-Yeast Tek and 79.3% for Yeast star.

Serological Tests

The effectiveness of the new Platelia Candida Antigen Plus (Ag Plus) and Antibody Plus (Ab Plus) assays (Laboratoires Biorad, France) were assessed using serum samples obtained from 21 candidiasis patients. In the Individual patient trial, the sensitivity of the Ag Plus and Ab Plus assays varied between 55-70% and 30-64% for patients with neutropenia for less than 15 days [33]. The isolation and detection time of these new tests is not substantially and better than in comparision of the traditional techniques for Platelia Candida. Lack of standard in the nucleic acid amplification techniques, commercial tests available for detection of t 1,3- β-D Glucan, and Mannan Antigen and Cand-Tec Candida antigen in serum [34]. For improvement of early diagnosis of IC, several serological tests for detecting fungal biomarkers (D.N.A., antigen and specific antibodies) are present. However, only a few tests are used for routine diagnosis of Candidemia, i.e. 1,3 β-D- Glucan, Mannan, Anti-mannan [26]. Carbohydrate is the most important element of the fungal cell wall; its transmembrane enzyme produces 1,3- β-D- glucan [24]. Sensitivities and specificities are 77%, 85% for BDG, 58%, 93% for mannan Ag, and 64%, 58% for CA respectively for diagnoses of Invasive Candidiasis [34]. The sensitivity of mannan antigen was less for Candida parapsilosis, and Candida guilliermondii fungemia than other species (P=0.005 and P= 0.046) and both species were not detected by Platelia Candida Ag Plus kit [34]. Surveillance cultures can be helpful in identifying patients who are at higher risk of developing aggressive candidiasis. However, little is known about the effect of candida colonization on the serum levels of diagnostic biomarkers [35]. The extent of Candida colonization in pediatric cancer patients and its possible effects on serum levels (1-3), βD glucan (BDG), Candida mannan [35].

Molecular Methods for diagnosis

Modern molecular application tools for the identification of fungal are modernizing faster than other ideal areas of clinical mycology. Due to the rapid accumulation of protein and DNA sequence data, the development of this area has been made possible and is continuing and accelerating with technology and the growing need. PCR is considered to be crucial for many methods like

the primary diagnostic strategy or used in the preliminary steps during the diagnostic assay. Many of the latest methods were used to develop through different molecular techniques that are matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, in house DNA- based techniques, peptide linked in situ hybridization and commercial polymerase chain reaction [1].

Polymerase Chain Reaction

Molecular work for fungal infection identification depends on culture purity. For early initiation of antifungal therapy and better therapeutic results, detection of fungal DNA detect by PCR is more rapid and sensitive than the conventional methods, which are time-consuming and less sensitive [36].

Nucleic acid based (NA) detection plays an important role in the identificationof fungal analytes by providing sensitive and specific bioassays. The method is based on three distinct reactions that use thermal cycling to amplify DNA [37]. Denaturation that transforms double-stranded DNA into a single stranded template under extreme temperature conditions, [38] Annealing a single-stranded DNA template at a stringent annealing temperature that fits a specially formulated primer, and [21] polymerase [33]. The extension used enhances the activity of the polymerase reaction by creating new chains that are complementary to the single-chain sequence. Different PCR techniques focused on different applications such as multiplex PCR (for the identification of different analytes in a samples), real- time PCR (for monitoring of amplification in real- time format), reverse transcription enzyme PCR (RT-PCR) etc [33]. It is in development. Nested PCR to increase specificity by amplifying the RNA template and two primer pairs to avoid unexpected primer binding and non-specific amplification Conventional PCR fungal ribosomal genes are more common DNA. target for identification of fungal species [14]. NA-based detection or molecular diagnosis may be used on the basis ofhybridization, microarray and amplification techniques. Sample preparation is one of the most difficult stages in the use of these techniques [33]. This involves DNA extraction and purification steps to eliminate intracellular DNA, target DNA concentration and potential inhibitor, debris and contaminants from the fungus. Sample preparation, appropriate primer, DNA target selection, extraction of DNA and amplification is directly dependent for the sensitivity of DNA based assay [39]. In Clinical isolates Lanosterol alpha–demethylase (L1A1) gene was first described by PCR amplification for detection of Candida albicans [32]. Morace described appropriate restriction enzyme analysis (R.E.A.) for detection of 350 bp segment of the P-450 lanosterol 14 α demethylase gene for diagnosed Candida species involved in human infections [35]. 5.8S, 18S and 28S ribosomal RNA genes are the highly conserved sequence for specific identification of Candida species, and Internal transcribed spacer regions (ITS) are highly conserved as well as variable region are located between these genes for identification of Candida albicans [39]. A microarray for the identification and detection of 12 spp. of Candida and Aspergillus [33]. Oligonucleotide probes have been designed to detect the internal transcribed spacer (ITS) region of the rRNA gene. The Universal primers ITS1(TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) for amplification of ITS rDNA. Identification of Fungal Isolates is also confirmed by the amplification of the D1-D2 region of the 28S rDNA using NL1 (GCATATCAATAAGCGGAGGAAAAG) and NL4 (GGTCCGTGTTTCAAGACGG) primers [18]. Most suitable yeast specific universal primer U.N.I. 1 (5'-GTCAAACTTGGTCATTTA-3') and U.N.I. 2 (5'-TTCTTTTCCTCCGCTTATTGA-3') was used to amplify the internal transcribed spacer regions 1 (ITS1) and (ITS2) including 5.8S RNA, based on ITS1 and ITS2 species-specific primers Calb, Ckru, Cgla, Cpar, Ctro, Cgui, Cdub, Clus was used for identification of eight clinically significant Candida species DNA amplification Candida albicans, Candida krusei, Candida glabrata, Candida parapsilopsis, Candida tropicalis, Candida guilliermondii, Candida dubliniensis, Candida lusitaniae respectively [32]. Standard identification of rDNA gene sequence analysis considered with D1-D2 region or internal transcribed regions [18].

Candida auris detected by sequence analysis of the ITS region and D1/D2 ribosomal DNA domains (rDNA) [40]. PCR-RFLP using restriction enzyme, identification of clinical isolates of various Candida species which were previously identified by phenotypic methods is used [34]. Multidrug-resistant species Candida auris is correctly characterized by internal transcribed spacer (ITS) and 28S rDNA gene considered as a standard gold method for identification [4]. The DNA microarray technique has become a viable medium for the identification of Candida. Thousands of complex gene probes are placed on solid substrate surfaces, e.g. Wafers of silicone, glass or nylon [33]. These investigations are planned to target the DNA sequence of the genome. Next, the series will be searched by the mark (fluorescent or radioactive label). When the sample reaches the microarray the hybridization occurs either in bulk or with the aid of any liquid handling instrument, such as microfluidics. The surface of the structure is then scanned to see the fluorescent pattern and the sample is measured using a microarray reader. Several microarrays have been developed to detect candida [33].

Matrix-Assisted Laser Desorption/ Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS is a fast and reliable diagnostic tool based on non-nucleic acid sequence, and it works by evaluate the mass to charge values and matches them with observation of protein molecule of the organism [46,48]. Presently, MALDI-TOF/MS help to recognize pathogenic bacteria and fungi frequently from positive blood cultures and can also directly used to clinical specimens at the genus level [1]. This instrument consists of an ion source that moves the sample molecules to the gas phase, a mass analyzer that resolves ion based on mass-to-charge ratio and a portion that detects the ions [4]. Recently, clinically important Candida species identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) with efficiency and accuracy. Out of 1383 isolates, 950 isolates (68.7%) detected by traditional method and accurate identification was performed by MALDI-TOF system in which 836 isolates of Candida albicans, 44 of Candida krusei and 39 of Candida tropicalis, 31 of Candida dubliniensis were identified. 433 (31.3 percent) isolates were eventually identified by a sequence of r DNA [18]. This approach is advantageous because it takes

less time to classify after a positive blood Culture has ever been achieved, it demonstrates the potential to detect uncommon Candida species [1]. This instrument has a high- cost set yp, useful data needed, poor sensitivity for direct testing of whole blood samples and misidentification of mixed culture samples [1].

Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA-FISH)

FISH is a technique that uses oligonucleotide fluorescent probes that bind to the genes found in the genome of the cell [33]. One of the most reliable approaches to diagnose infection with candida is Accurate fluorescent staining of tissue samples based on immunechemistry and genetic markers is established [33]. To separate Candida, the FISH technique was used, Candida parapsilosis attributable to Candida albican with probes that target the 18S rRNA region of Candida. Efforts have also been made to resolve these difficulties by the use of various DNA based probes with unlabelled helper probes. When different DNA-based FISH probes (Calb-1249 or CalB2208) are used, the helper probes Candida albicans also greatly increased the intensity of fluorescence [33].

PNA- FISH, a molecular technique detects the fluorescent colours. It uses dual colour labelled fluorescent DNA to target 26S r RNA sequences of Candida species [1]. Green fluorescence is given by Candida albicans and Candida parasilosis, yellow by Candida tropicalis and red by Candida glabrata & Candida krusei [1].

PNA FISH Flow Kit tested by using isolates were hybridized with PNA-based probes which identified multiple Candida species including Candida glabrata, Candida albicans, Candida tropicalis, Candida krusei, Candida parapsilosis, Candida dubliniensis, Candida kefyr [33]. The specificity and sensitivity of the PNA FISHFlow probes were 100% (49 out of 49) and 100% (101 out of 101) for identification of Candida and the entire staining process was completed within 1 hour. Gherna et al. also used the AdvanDX PNA FISH detection kit and identified Candida albicans and Candida glabrata from 40 positive blood culture vials within 1.25 hours [33]. Overall, the techniques of FISH detection provide a fast forum for the precise identification of Candida species from blood culture. While it provides high sensitivity and precision, it is not possible to use FISH directly with blood samples [33].

Recent details of studies on Candida in India.

Fatima et. al. conducted a study in 2017 on 90 consecutive clinical isolates from NICU in India. There were 90 isolates from blood of neonates out of which all 90 samples were positive. In this study Candida krusei was found 48(53.33%) and followed by Candida parasilopsis, Candida albican, Candida lusitaniae, Candida tropicalis, Candida glabrata and Candida guilliermondii with specificity 12(13.33%), 11(12.22%), 8(18.88%), 5(5.55%), 4(4.44%) and 2(2.22%) respectively. PCR-RFLP restriction enzyme Mspl give bands at Candida albican (297,386bp), Candida krusei (261,249bp), Candida tropicalis (340,184bp), Candida glabrata (557,313bp), Candida lusitaniae (266,117bp) &Candida guilliermondii (371,155,826bp). All strains were sensitive to fluconazole, amphoterecin B, voriconazole, caspofungin, flucytosin except 25% of C. glabrata and all isolates of C. krusei were resistant to fluconazole & moderately sensitive to voriconazole.

In 2015, Dewan Eshani et. al. done prospective observational study in haematological malignancies at Himalayan region of northern india. Total 150 patients with 40 age group enrolled with fever (febrile neutropenia) 50 blood culture were positive. Highest prevalence (46.67%) of Candida tropicalis was found in AML & ALL. The ration between Candida albican and Candida non albican was (1:2.75). Candida krusei and Candida tropicalis were resistance to azoles and Candida albicans, Candida parasilopsis, Candida dubliniensis sensitive to azoles.

Gamaletsou et. al. developed a study in Greece which is population based prospective multicenter . They were comparing between patients with hematological malignancies and without hematological malignancies. 40 clinical isolates with hematological & 967 isolates without hematological malignancies were positive. Itraconazole were resistance for all tested Candida species.

In 2010, Zhoa et.al. conduct a study on critically ill 589 pediatrics patients with hematological malignancies. Serological tests were done for identification of Candidemia by beta-D-glucan assay &GKT-5M set kinetics fungus detection kit/plus. Candida glabrata and Candida tropicalis were mainly detected in this study.

A population based prospective study in 2010 by Milkulska et. al. on total 453 leukemia patients & 767 controls for detection of Mannan and Antimannan in serum by ELISA (Platelia; Bio-Rad Laboratories Marnes La Conquette France). Senisitivity & specificity of Mn 58% & 93% respectively. Antimannan sensitivity & specificity was 59 & 83% respectively. Combined Mn/AMn sensitivity 83% & specificity 86%. Prevalence of Candida albican were high.

Target Site of Antifungals

Polyenes, fluoropyrimidines, echinocandins and azoles are the group of drugs being used as a commercial antifungal in the treatment of Candidemia infections [3]. To diagnose early detection of candida infection is still a problem [3].

In clinical treatments, only a few types of antifungal drugs are available in the market for the treatment of Candida species infection. The target site of these antifungal classes are azoles (Inhibitor of lanosterol 14 –α- Demethylase), echinocandins (Inhibitor of 1,3, β – D- glucan synthase), polyenes (Bind with ergosterol and disrupt the major lipid component of the fungal cell membrane), Nucleoside analogue (Inhibitor of DNA/RNA synthesis) and some other antifungal agents are Allylamines, thiocarbonate (disrupt cell membrane by inhibiting the biosynthesis of ergosterol & squalene epoxidase) and Griseofulvin (disrupt spindle & cytoplasmic microtubule production, inhibit fungal mitosis) [41].

Reference	Area	Isolates tested	Antifungal susceptibility result	Antifungal susceptibility result				
				(percentage resistance)				
				VRC	FLU	ITR	AMB	Echinocandins
Kaur et. al. 2020	Chandigarh	7927	BMD	2.4	8.5	5.1	3.2	CAS 6.2;ANI 2.4; MIC 3.04
Nazir and Masoodi 2018	Kashmir	80	VITEK 2	0	36.3	NA	18.8	NT
Thomas et al. 2016	Ludhiana	105	Disk Diffusion (NCCLS M44A)	NT	42	69	NA	NT
Bhattacharjee 2016	Kolkata	64	VITEK 2	8.82	NA	17.6	44.1	NT
Chaudhary et al. 2015	Haryana	100	Disk Diffusion	NT	--	--	0	NT
Chakrabarti et al. 2015	Multicentric	1400	BMD	5.6	6.2	1.2	2.1	CAS 5.6;ANI 1.7; MIC 1.7
Mathur et al 2014	Delhi	212	VITEK 2	NT	3.3	NT	3.3	NT
Juyal et al 2013	Uttarakhand	132	Disk Diffusion	NT	34.1	26.5	3.8	NT
Adhikary and Joshi 2011	Karnataka	60	VITEK 2	0	25	NT	8	NT
Kotwal et al 2011	Uttarakhand	96	Disk Diffusion, BMD	NT	26	18.7	0	NT
Kothari and Sagar 2009	Delhi	96	BMD	44	64	76	8	NT

Table 1 : Antifungal resistant Details reported from India [49]

BMD: Broth microdilution, VRC: Voriconazole, FLU : Fluconazole, ITR: Itraconazole,

AMB: Amphoterecin B, CAS: Caspofungin, ANI: Anidulafungin, MIC: Micafungin,

NCCLS: National Committee for Clinical Laboratory Standard, NA: Not Tested,--Only species wise resistance is known

Conclusions

Invasive Candidiasis is one of the emerging diseases which have a high mortality rate; therefore, the role of antifungal therapy is a must. Delay in antifungal therapy due to lack of sensitiveness of gold standard blood culture technique. Rapid and accurate diagnosis of fungal infections is provided by PCR technology due to which initiations of therapy are possible. Molecular methods have been processed and developed with varying sensitivity and specificity for detection of Candida and identification of their species directly from blood samples. In Laboratories, a molecular-based diagnostic is the primary source of identification method and is facing several challenges for routine samples. There is an urgent need to develop some potent alternative of the azoles that could be more effective and efficient as compared to the already used drugs.

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