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Candidiasis: Past, present and future

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ABSTRACT

Background: Until 19th century most of infectious diseases were attributed to bacterial, viral and parasitic origin. The role of fungus in infection was rarely documented. **Aim:** In this review, the past, present and future aspect of candidiasis with reference to biology, epidemiology, pathogenicity, laboratory diagnosis and antifungal resistance is discussed. **Methods:** Published articles were reviewed. **Results:** Over the last few decades, the scenario of infectious diseases has changed and fungi, which were once studied only as "microbiological curiosities" with less or no pathogenic role have emerged as important cause of opportunistic and health-care associated infections. Among various mycotic infections, candidiasis has greatest effect due to its frequency and severity of complications. **Conclusion:** As the epidemiology of candidiasis is not constant, the incidence rates, species distribution and even antifungal susceptibility profiles seems to be changing. Increased incidence of candidiasis along with emergence of treatment resistant NAC spp. has become an important healthcare issue worldwide. Therefore, the early and accurate identification of *Candida* spp along with susceptibility testing is very important for timely institution of appropriate therapy and prevention of emergence of drug resistant *Candida* spp.

Key words: Antifungal resistance, *Candida albicans*, disseminated candidiasis, drug resistance, mucocutaneous candidiasis, non *albicans Candida* species

INTRODUCTION

Even in the era, where the field of Medicine has progressed beyond leaps and bounds, infectious diseases remain one of the most significant threats to mankind. Among various infectious diseases, fungal infections are increasing in incidence and drug resistance. The severity of mycoses ranges from moderate to fatal and is dependent on site of infection and immune status of the host.[1] The common targets of fungal pathogens are HIV/AIDS and cancer patients, transplant recipients and drug abusers.[2]

Although the target group of mycotic infections is limited as compared to viral or bacterial counterparts, these infections are often associated with high morbidity and mortality rates.[1] High morbidity and mortality rates associated with mycoses may be due to lack of rapid sensitive diagnostic techniques, emergence of drug resistance and dearth of effective antifungal drugs and vaccines. Among various mycotic

infections, candidiasis has greatest effect due to its frequency and severity of complications.[3]

Candida spp. is the only pathogenic fungus which is capable of causing a wide range of clinical manifestations from mucocutaneous overgrowth to life threatening disseminated infections.[4] Although *C. albicans* is considered as, the most pathogenic member of the genus and the leading cause of all forms of human candidiasis, more recent studies documents a shift towards treatment resistant non *albicans Candida* (NAC) species.[5-7]

As the epidemiology of candidiasis is not constant, the incidence rates, species distribution and even antifungal susceptibility profiles seems to be changing.[8] The changing trends of candidiasis underscore the need of continuous monitoring and up to date knowledge for effective prophylaxis and treatment. In this review, the past, present and future aspect of candidiasis with reference to biology, epidemiology, pathogenicity, laboratory diagnosis and antifungal resistance is discussed.

LITERATURE REVIEW

General characteristics of genus *Candida*

The genus *Candida*, belong to the Phylum Ascomycetes, Class Blastomyces, Order Cryptococcales, Family Cryptococcaceae.[9] It includes characteristically white asporogenous yeast capable of forming true or pseudohyphae (except *C. glabrata* and *C. parapsilosis*. *C. glabrata* is incapable of forming both true hyphae and pseudohyphae whereas; *C. parapsilosis* does not produce true hyphae, but can generate pseudohyphae).[10, 11]

Pseudohyphae are formed from yeast cells or hyphae by budding but this bud doesn't detach from the parent cell.[10,11] The new growth further elongates and form filaments with constrictions at cell-cell junctions.[11] Pseudohyphae lack internal cross walls (septa).[11] True hyphae are formed from yeast cells or even as branches of existing hyphae. Its development is initiated by a 'germ tube' formation. This germ tube elongates and then branches with defined septa dividing the hyphae into separate fungal units.[11]

C. albicans and *C. dubliniensis* are considered as truly polymeric because its ability to form hyphae and/or pseudohyphae.[11] They can also form germ tube within 2 hrs when inoculated in human serum or other substances.[12] Therefore positive germ tube test or Reynolds Braude's phenomenon is a diagnostic feature of these two species.[11,12] Yeasts belonging to the genus *Candida* are oval, elliptical or cylindrical, unicellular or bicellular measuring 2-5x3-7 µm and has double layered cell wall.[3]

The genus *Candida* contains approximately 200 species, but only few have been implicated in human infection.[3] Table 1 shows, the *Candida* spp. commonly associated with human infections. The members of the genus are mostly saprophytic and few as commensals of human skin and various mucus membranes. *Candida* colonization begins soon after the birth and persists throughout life.[3]

Epidemiology

Candida spp. are ubiquitous and can exist in both saprophytic and commensal state. Numerous factors contribute the transition of *Candida* from a commensal to a potent pathogen.[13] These include both host predisposing factors and pathogenicity of infecting species. Table 2 shows the important host factors predisposing to candidiasis.

The expanding population of immunocompromised patients, the increasing use of broad spectrum antibiotics, cytotoxic chemotherapies and transplantations are among important factors predisposing to candidiasis.[14]

Candida spp. can elicit infections in immunocompetent as well as immunocompromised individuals but the incidence of candidiasis is more in immunocompromised hosts. Therefore candidiasis can be rightly called as "disease of diseased".[15] As discussed earlier, *Candida* spp. causes skin, mucosal and invasive infections.

Superficial and mucocutaneous candidiasis is extremely common and can occur in otherwise healthy individuals. It includes common mucosal infections, such as thrush, vulvovaginal candidiasis (VVC), cutaneous candidiasis, onychomycosis and chronic mucocutaneous candidiasis.[3] These infections are quite specific, usually self limiting in immunocompetent hosts and easy to treat with basic hygiene measures and local treatment.[16]In case of immunocompromised individuals, mucocutaneous candidiasis should be given a special attention as these infections could become a gateway to systemic spread. Candidiasis, especially oropharyngeal candidiasis (OPC) is the most common opportunistic fungal infection among people living with HIV/AIDS (PLHA).[17] It develops in 80-90% of HIV infected patients at some point during progression of their disease.[17] At many times it may be the 1st visible sign of HIV infection and the patient's only chief complaint. Therefore,

it can be rightly said, "*Candida* is a better physician, as it can discover abnormalities in person's immune system much sooner, than we with our diagnostic tests". Although, the incidence of OPC in PLHA has decreased dramatically with the introduction of high active antiretroviral therapy (HAART), it is being still reported from many poor and developing countries. Episodes of OPC and esophageal candidiasis are also seen in HAART treated HIV infected patients with low CD4+ cell counts.[17]

VVC is among the common clinical condition in women seeking gynecological care; approximately about 75% of women experience at least one episode of VVC.[18] It is very frequently associated with pregnancy, diabetes mellitus, obesity or corticotherapy.[18] In case of diabetes, the high glucose content of the tissue enhances *Candida* colonization and infection. In pregnancy, estrogens favor *Candida* colonization and infection by enhancing the glycogen content of vaginal epithelial cells.[18]

Disseminated candidiasis (DC) is a devastating disease associated with high morbidity and mortality rates. [8] It refers to conditions where, *Candida* invasion is shown from cultures or histology results at non-adjacent, normally sterile sites.[19] DC is usually associated with insufficiencies in host defense that commonly occur in hospitalized, cancer and ICU patients receiving chemotherapy and broad spectrum antibiotics.[20]

Candida blood stream infection or candidemia represents 10-20% of all candidiasis and is considered as the tip of the iceberg of infections due to *Candida* spp.[18] *Candida* is considered as the fourth leading cause of health care associated or nosocomial blood stream infection and the number three cause of blood stream infections in the ICUs.[20] Candidemia is usually associated with high mortality rate especially in ICU patients.[8] It also increases the hospital stay and health care cost.[8] Surgical and ICU patients are at higher risk of nosocomial fungal infections. In ICU patients, the most common types of *Candida* infections are BSI, catheter-related infections, intra-abdominal infections and urinary tract infections.[19]

Only a decade back, DC used to be a rare infection restricted to burn and patients with severe trauma. In recent years, the increasing population of patients in need of chemotherapy, organ transplants and intensive care has increased the incidence of DC. The incidence of *Candida* blood stream infection is usually higher in the overall hospital than in the general population but much lower than among cancer and seriously ill patients.[8]

Although *C. albicans* used to be the predominant isolated species from various clinical specimens, recently published research highlights an increase of NAC spp., such as, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. dubliniensis*.[6,8,11] They are emerging as both colonizers and potential pathogens.[11] Pfaller and Diekema (2007) in the study on epidemiology of invasive candidiasis observed that *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis* collectively accounted for approximately 95% of identifiable *Candida* infections.[14] The apparent increased isolation of NAC spp. from various types of candidiasis may related to improvements in diagnostic methods, such as the use of chromogenic media and the introduction of molecular diagnostic techniques. The high prevalence of NAC spp. could also be related to empirical use of antifungal drugs for prophylaxis and treatment of fungal infections.

C. tropicalis is one of the most commonly isolated NAC spp.[13] It is most commonly isolated from ICU and cancer patients. *C. tropicalis* has higher potential of dissemination in neutropenic patients compared with *C. albicans* and other NAC spp.[11] BSI due to *C. tropicalis* is often associated with high mortality rates. *C. tropicalis* disseminates at high potency in neutropenic individuals compared with *C. albicans* and other NAC spp.[11]

Once considered as a nonpathogenic saprophyte, *C. glabrata* has emerged as an important pathogen.[15] It is the second or third most common *Candida* spp. associated with different types of candidiasis.[11] Baddley *et al* (2001)[21] and Malani *et al* (2005)[22] reported *C. glabrata* as to be the most common isolate from candidemia cases. It is the second most common *Candida* spp. responsible for blood stream infections in United States.[15] The incidence of *C. glabrata* fungaemia is higher in adults as compared to neonates and children.[11] The mortality rate associated with *C. glabrata* infections is highest as compared with other NAC spp [11] The risk factors for *C. glabrata* has emerged as prominent pathogen among patients with haematological malignancies and bone marrow transplants.[23]

C. parapsilosis is the second most commonly isolated *Candida* spp. from normally sterile body sites of hospitalized patients.[11] It is one of the most commonly isolated fungi from human hands.

Table 1: *Candida spp* of medical importance

***Candida* spp.**	**Clinical manifestations**
C. albicans	Broad spectrum of clinical manifestations ranging from mucocutaneous growth to disseminated infections.
C. tropicalis	Candidemia and other disseminated infections in immunocompromised patients.
C. glabrata	Candidemia, disseminated candidiasis, candiduria.
C. krusei	Candidemia, endopthalmitis, diarrhea in neonates.
C. parapsilosis	Candidemia, medical-device associated infections, infections related to contaminated solutions.
C. kefyr	Disseminated candidiasis.
C. dubliniensis	Oropharyngeal candidiasis in HIV/AIDS patients.
C. famata.	Candidemia.
C. rugosa	Candidemia.
C. lipolytica	Intravenous catheter-associated candidemia.
C. norvegensis	Infections in renal transplant recipients.
C. lusitaniae	Candidemia and other disseminated infections.
Data derived from references: [4, 8, 11, 19, 25, 27]	

Table 2: Host factors predisposing to candidiasis

Predisposing factor	**References**
➢ HIV/AIDS.	[2, 3, 4, 13,14]
➢ Diabetes.	[4,6]
➢ Extremes of ages.	[13]
➢ Malignancy.	[2, 13]
➢ Bone marrow transplant.	[2, 13]
➢ Solid organ transplant.	[2,13]
➢ Long hospital stay.	[2,4,13]
➢ Treatment with broad spectrum antibiotics	[2,4,6,8,13,14]
➢ Neutropenia	[2,4]
➢ Indwelling medical devices	[2,4,6]

hyperalimentation solutions and is able to colonize intravascular devices and prosthetic materials.[11] In contrast to other NAC spp. *C. parapsilosis* infections is associated with drastically high mortality rate in low birth weight neonates.[11] *C. krusei* was considered to be a facultative saprophyte and a transient human commensal.[11] However, in recent years there has been a marked increase in the reports of *C. krusei* as an emerging human pathogen with a spectrum of clinical manifestations such as candidemia, endopthalmitis, arthritis and endocarditis.[24] *C. krusei* infections are usually common in hospitalized patients.[25] The advent of HIV/AIDS and the widespread use of fluconazole both for prophylaxis and treatment of mycotic infections in these patients have contributed significantly for rise in *C. krusei* infections.[25] *C. dubliniensis* once only associated with OPC in HIV/AIDS patients has been recently isolated from other body site/specimens like vagina, urine, skin and gastrointestinal tract of HIV negative patients.[4] This pathogen rarely causes DC as it lacks genome important for hypha-related virulence genes and has a limited ability to undergo yeast-to hyphal transformation.[4]

As concern regarding the high incidence of infections caused by NAC spp. and the emergence of antifungal resistance is rising future research must be directed towards epidemiology of NAC spp. in different health care set ups.

Pathogenicity

Candida infections are mostly endogenous.[26] However, exogenous transfer may occur in health care setup due to contaminated medical devices and the hands of health care personnel. *Candida* spp. survives for upto 4 months in the hospital environment.[27] The major steps in the pathogenesis of candidiasis include (i) increased *Candida* colonization due to treatment with broad spectrum antibiotics (ii) disruption in skin and mucosal barrier (iii) immunodeficiencies either induced or acquired.[27]

Previously, the role of *Candida* in establishment and progression of infection was considered to be passive. Therefore, organic weakness or an immunocompromised status of the host was considered the only mechanism responsible for the establishment of opportunistic infection.[4] Recently, this concept is altered and it can be stated that, *Candida* can actively participate in the pathophysiology of the disease progression using mechanisms of aggression called virulence factors.[4] Virulence factors are all traits required for establishment and progression of infections.[11] These factors interact directly with host cells causing damage.[28]

The pathogenicity of *Candida* spp. is attributed to number of virulence factors, like adherence to host tissues and medical devices, biofilm formation and the secretion of hydrolytic exoenzymes.[5, 6, 13] The combination of different virulence factors contributes at each stage of infection.

Adherence to host tissues is the most critical step in the process of *Candida* colonization and infection.[13] It prevents or at least reduces the extent of clearance by the host defense mechanisms.[29] Adherence to host tissues also ensures the delivery of toxins and enzymes to the host cells. In addition, binding to the host tissues helps *Candida* to penetrate and disseminate. *Candida* cells can adhere to variety of host cells including epithelia, endothelia and phagocytic cells. Adherence of *Candida* cells to host tissue is mediated by non specific factors (hydrophobicity and electrostatic forces) and is progressed by specific adhesins present on the surface of *Candida* cells that recognize ligands such as proteins, fibrinogen and fibronectin. [30] Adhesins specifically bind to amino acids and sugars on the surface of host cells. It also promote adherence to abiotic surface.[4]

Due to their versatility of adapting to a variety of habitats including various medical devices, *Candida* spp. have emerged as one of the most important cause of health care associated infections (HCAIs). *Candida* spp. can form biofilm on most, if not all, medical devices. Medical device associated infections are common in ICUs.[31, 32] Biofilms are specific and organized surface-associated communities of microbial cells embedded within extracellular matrix.[33, 34] Biofilm matrix of *C. albicans* is mainly composed of carbohydrates, proteins, phosphorus, hexosamines and uronic acid.[34] The available literature has revealed a dearth of information about the biofilm matrix composition of NAC spp.[34] Extracellular matrix of *C. parapsilosis* biofilms contains large amount of carbohydrates with correspondingly low protein levels.[34] Biofilm matrix of *C. glabrata* has high levels of both proteins and carbohydrates while *C. tropicalis* biofilm matrix contains low levels of proteins and carbohydrates compared with the other NAC spp.[34] Biofilm formation can be affected by number of factors like nature of substratum, nutrients, presence of body fluids, availability of oxygen, extracellular polymeric substances and *Candida* spp.[33]

C. albicans biofilm formation has been studied more extensively as compared to NAC spp.[33] It involves 3 three overlapping phases:[33, 34] (i) Early phase (0-11 h):- Adherence of yeast cells to the

surface of device. (ii) Intermediate phase (12-30 h):- Formation of a matrix with dimorphic switching, that is, conversion from yeast phase to hyphal form. (iii) Maturation phase (38-72 h):- Increase in the matrix material taking on a three-dimensional architecture.

Medical device-associated candidiasis has high mortality rates. *Candida* biofilms are resistant to many antifungal agents and therefore removal and later replacement of the infected device is very essential for treatment.[4] Biofilm confers significant resistance to antifungal drugs by limiting the penetration of substances through the matrix.[34] It also protects yeast cells from host immune response. Biofilm formation was observed previously only among *C. albicans* isolates. Interestingly, recent studies have reported greater biofilm-forming ability in NAC spp like *C. tropicalis* and *C. glabrata*.[6,13,15] This observation underscores the importance of further research, as NAC spp. are now considered as important emerging pathogens worldwide. In our recent study, *C. tropicalis* demonstrated high biofilm forming ability as compared to *C. albicans*.[6]

In *Candida* spp, production of extracellular hydrolytic enzymes is one of the important attributes contributing to pathogenicity. Extracellular hydrolases play an important role in adherence, tissue penetration, invasion and the destruction of host tissue.[11] Production of extracellular hydrolytic enzymes varies among the species and also depends on the source or site of infection. Secreted hydrolytic enzymes in *Candida* spp. include phospholipase, lipase, phosphomonoesterase, hemolysins, hexosaminidase and secreted aspartic proteinase. Phospholipases and proteinases are the most important and most studied hydrolytic enzymes of *Candida* spp.[4]

Phospholipases hydrolyze phospholipids into fatty acid and also expose receptors on the host cell membrane to facilitate adherence. The secretion of phospholipases by *C. albicans* was 1st reported by Costa *et al* and Werner.[35] Till now, seven phospholipase genes have been identified (PLA, PLB1, PLB2, PLC1, PLC2, PLC3 and PLD1); but only four of these (PLB1, PLB2, PLC1 and PLD1) has been well characterized.[4] The exact role of these enzymes in the pathogenesis of candidiasis is still unclear.

Early studies showed that only *C. albicans* produces extracellular phospholipase. However, recent studies have demonstrated that NAC spp. can also secrete phospholipase. In our study on NAC spp. high phospholipase activity was noted in *C. tropicalis* and *C. glabrata* whereas, expression of phospholipase enzyme was low in *C. dubliniensis*.[6] Low phospholipase activity could be one of the possible reason for minimal or no ability of *C. dubliniensis* to cause invasive infections.[6] Phospholipase production was high in biofilm producing *Candida* isolates. Therefore screening of phospholipase activity in biofilm forming *Candida* spp. can serve as an important parameter to differentiate invasive strains from non invasive colonizers.[36] Ying and Chungyang demonstrated co-relation between high phospholipase activity and resistance to antifungal drugs.[37] Therefore, phospholipase may play an important role in the emergence of azole resistance.[37] The quantity of phospholipase production also varies with the specific isolate and the site of infection. Isolates from blood stream infections generally demonstrate high phospholipase activity as compared to those from wound or urine.[38] More extensive research is required to explore the role of these enzymes in the pathogenesis of candidiasis.

Secreted aspartyl proteinases (Saps) degrade epithelial and mucosal barrier proteins such as collagen, keratin and mucin.[39] The extracellular proteinase of *C. albicans* was first described by Staib.[39] Saps are also capable of degrading complement, cytokines and immunoglobulins.[40]

Saps are encoded by 10 SAP genes.[41] Each Sap encoded by different gene has different role in the pathogenesis of candidiasis. Sap1 to Sap6 facilitates adherence, tissue damage and evasion of host immune mechanisms.[42] The role of Sap7 to Sap10 remains unclear, but there is evidence that Sap9 and Sap10 are not exoenzymes as they are not secreted from the cell. Sap9 and Sap10 are regulatory proteinases that are important for maintaining cell surface integrity.[42] Saps of pathogenic *Candida* spp. have been extensively studied. *C. albicans*, *C. dubliniensis*, *C. guilliermondii*, *C. parapsilosis* and *C. tropicalis* possess SAP gene families.[43] *C. dubliniensis* isolate often are more proteolytic than *C. albicans*.[44] Information on Saps of other NAC spp. like *C. glabrata*, *C. krusei* and *C. kefyr* is limited.[43]Although very little is known about proteinase production in *C. glabrata*, few recent studies have reported the capability of *C. glabrata* to produce proteinase.[6, 15] Therefore, future research on proteinase activity of *Candida* spp. should be directed towards these species.

Haemolysins are considered as one of the important virulence factors enabling *Candida* survival and persistence in the host.[11] It degrades haemoglobin and facilitates recovery of the elemental iron from host erythrocytes.[11]

Haemolysin production is affected by the presence of glucose in the culture medium. Luo *et al.* (2001) detected only alpha but no beta haemolysis on glucose free sheep blood agar.[45] *In vitro*, haemolysin activity is demonstrated by *C. albicans* and other species of *Candida* from NAC group.

Coagulase activity is one of least studied virulence factor of *Candida* spp. Enzyme coagulase binds plasma fibrinogen and activities a cascade of reactions that induce clotting of plasma. Coagulase production in *Candida* spp. was first reported by Rodrigues *et al* (2003).[46]

The identification of virulence attributes unique to a particular *Candida* spp. is very essential to understand the pathogenesis and epidemiology of these infections and in future it may provide powerful insights for development of new antifungal drug targets.[6]

Laboratory diagnosis

Laboratory diagnosis of candidiasis is hampered by two main reasons, the commensal nature of the organism and the lack of rapid and sufficiently sensitive or specific diagnostic techniques. As in any other infectious disease, the laboratory diagnosis of candidiasis depends on the infection caused by it.

In case mucocutaneous candidiasis, the laboratory diagnosis is comparatively easy and involves: - direct demonstration (budding yeasts, pseudohyphae or hyphae) in clinical specimens and isolation of *Candida* in culture followed by species identification. Direct microscopic examination is simple, rapid and cost effective method for diagnosis of candidiasis.[47] The demonstration of pseudohyphae along with yeast cells is an important diagnostic feature to distinguish infection from colonization.[47] But as *Candida* spp. is a commensal of oral cavity and vaginal mucosa, both its demonstration in direct smear and isolation in culture is essential for establishment of diagnosis.[47]

Candida grows on most laboratory media used for isolation of fungi. Sabouraud dextrose agar (SDA) is the most commonly used medium for isolation of *Candida* spp. from various clinical specimens.[48] On SDA, *Candida* colonies are cream to yellow in color. Colony texture is dependent on the species and may be smooth, glistening or dry, or wrinkled and dull.[47] SDA is not selective medium, however addition of antibiotics (chloramphenicol, gentamicin and/or tetracycline) and cycloheximide avoids the bacterial and saprotrophic fungal contamination.[48, 49]

Various differential media are available for identification of *Candida* spp. from clinical specimens. These include Pagano-Levin agar, phosphomolydate agar, Nickerson's medium and chromogenic media like CHROM agar.[47] Chromogenic media like CHROMagar *Candida* (figure 1) are widely used for primary isolation and species differentiation of *Candida* spp. from clinical specimens.[47] This medium is selective and differential for identification of *Candida* spp like *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. dubliniensis*. Species identification once seldom performed for *Candida* has become important due to emergence treatment resistant of NAC spp.[47]

Figure 1: CHROMagar *Candida* showing growth of different *Candida spp*.

The mycological workup for species identification of *Candida* starts with germ tube test.[12] Due to inherent safety problems associated problems associated with use of human serum, many diagnostic laboratories have started using non human serum media like egg white, saliva, sheep serum, peptone water and trypticase soya broth for testing of germ tube production.[12] Species identification can be done by carbohydrate assimilation and fermentation.[47] Various modifications of carbohydrate assimilation are now commercially available.[47] Examples of such commercially available kits include API-YI (Yeast indent) system/API-20C system, Auxacolor (Bio-Red), Uni-Yeast-Tek system, ID 32 C system, RapID yeast plus system (Innovative Diagnostic systems, Noncross) and VITEK system.[47] These kits are reliable for the identification of most frequently encountered *Candida* spp. Serological and molecular methods though available are not

standard routine procedure for diagnosis of superficial and mucocutaneous candidiasis.

Reliable and prompt diagnosis of disseminated candidiasis is important for initiation of appropriate therapy and successful outcome. Diagnosis of DC is often difficult due non-specific and complicated clinical presentation.[47] In most cases, invasive procedures are required for specimen collection.[47]

Historically, culture has been considered as standard diagnostic technique for DC.[50] However, *Candida* spp. may take weeks to grow in culture and the sensitivity of culture is also low.[50] Blood cultures are negative for *Candida* spp. in approximately 50% of autopsy-proven cases.[51] Blood culture methods using lysis-centrifugation tubes and automated monitoring systems have demonstrated moderate sensitivity.[51] The lysis-centrifugation system increases the yield of *Candida* spp. recovered from blood using a detergent to release fungi trapped within host phagocytic cells. In study of Berenguer *et al.*, the lysis centrifugation system was positive for only 43% of the autopsy-proven cases of DC.[51] Automated culturing systems like ALERT 3D and BACTEC 9240 have appreciable sensitivity and specificity for diagnosis of DC.[47]

In recent years, biomarkers and molecular techniques are gaining popularity for diagnosis of invasive fungal infections. These techniques involve minimal or no invasive procedures for sample collection and in most cases provide an earlier diagnosis. A Biomarker or biological marker is "a characteristic that is objectively measured and evaluated as an indicator of biological processes, pathogenic processes, or pharmalogic responses to a therapeutic intervention".[52] Galactomannan, 1-3-β-D-glucan, mannan and arabinitol are important fungal biomarkers.[50] Out of these, galactomannan and 1-3-β-D-glucan have been extensively studied and published over the last 20 years.[53] More research is needed to be conducted on newer surrogate markers, as in the next coming years detection of these markers may be looked upon as rapid and reliable techniques for diagnosis of DC.

Molecular based techniques can be recommended as rapid and sensitive methods for diagnosis of DC.[47] It can identify *Candida* isolates upto species level and most importantly, it can detect primary and secondary resistance to antifungal drugs.[54] However, many molecular techniques are expensive and require highly expertise technical support that may not be available in all microbiological diagnostic setup. Polymerase chain reaction (PCR) based assays are currently the most frequently used molecular technique for diagnosis of DC. Avni *et al.* reported, PCR to have good sensitivity and specificity for rapid diagnosis of DC.[55] As PCR is highly sensitive method, the chances of false positive results is often high.[47] Additional studies on PCR based methods either alone or in combination with other diagnostic methods should carried out to establish it's more precise and reliable application in diagnosis of DC.

Antifungal resistance

Antifungal drug resistance once rarely documented in *Candida* spp. has rised recently. The widespread empirical use of antimycotic drugs for prophylaxis and treatment, emergence of NAC spp. and progress in antifungal susceptibility testing methodology are important among various contributing factors.[56] Resistance to antifungal agents has serious implications for management of infections.

Antifungal resistance may be microbiological or clinical or combination of the two. Microbiological or microbial resistance is defined as the condition where the growth of infecting pathogen is inhibited by an antimicrobial agent concentration higher than the range seen for wild type strains.[56] It may be primary or secondary. Primary (intrinsic) resistance is innately present in some fungal species without prior exposure to the antifungal drug. [56] Example includes fluconazole resistance in *C. krusei*. Secondary (acquired) resistance develops among previously susceptible strains after exposure to the antifungal drug and is usually dependent on altered gene expression.[56] The development of fluconazole resistance in *C. albicans* and *C. dubliniensis* is an example for this type of resistance. Clinical resistance is defined as the condition where the fungal infection persists despite the administration of an antifungal drug with *in vitro* activity against the infecting organism.[56] Combined effect of various factors associated with the host, antifungal agent or pathogen contributes to clinical resistance.[56]

As discussed earlier, mucocutaneous candidiasis is very common and rarely fatal clinical manifestation of *Candida*. On the other hand, DC is associated with high morbidity and mortality rates.[56] Therefore this review is mainly focused on antifungal drug resistance in DC.

Antifungal drugs available for prophylaxis and treatment of invasive candidiasis include polyenes (amphotericin B deoxylate and 3 lipid formulation of amphotericin B), azoles (fluconazole, itraconazole, voriconazole and posaconazole), echinocandins (capsofungin, micafungin and

anidulafungin) and flucytosine.[57] These antifungal drugs differ in their mechanism of action, pharmacokinetic/pharmacodynamic properties, mode of administration, indication, cost and safety.[58] Therefore these factors must be considered while selecting a particular antimycotic drug to be administrated in a particular patient for a particular purpose. [58] The purpose of administration of antifungal therapy may be for prophylaxis, initial (empiric, preemptive, or targeted) therapy or salvage therapy.[59] Antifungal prophylaxis is initiated in patient without signs or symptoms of invasive mycoses, but is at sufficient risk of developing invasive mycotic infections.[58] These include recipients of hematopoietic stem cell or solid organ transplants, cancer and critically ill patients, immunosuppressed hosts or patients undergoing major surgery or treatment involving invasive medical devices. Primary antifungal prophylaxis involves the risks of overuse and also promotes the antifungal resistance.[60]

Several mechanisms have been proposed for antifungal resistance in *Candida* spp. These include (i) Alteration in the cell or plasma membrane. (ii) Efflux of antifungal drugs. (iii) Alteration in the affinity of the drug target. (iv) Sequestration of antifungal agent in cell organelle-like vacuoles. (v) Activation of alternate pathways leading to increased metabolism of the antifungal agent.[61] Resistance is combined effect of more than one of these mechanisms.

Many researchers have explored the molecular mechanisms responsible for azole resistance in *Candida* spp.[61] These mechanisms are mainly studied in *C. albicans* to lesser extent in NAC spp. such as *C. glabrata*, *C. tropicalis* and *C. krusei.* [61] As per these studies different cellular alterations are known to contribute the decrease of azole susceptibility.[61] Till now four distinct molecular resistance mechanisms are known.[61] These include 1) Failure of drug accumulation. 2) Alterations in Erg11p. 3) Upregulation of ERG11. 4) Alteration in sterol composition. Upregulation of multidrug transporter gene is the most mechanism for azole resistance in *Candida* spp. [61]

The emergence of antifungal resistance and gradual increase in the number of new and broad spectrum antifungal drugs has made antifungal susceptibility testing of *Candida* spp. important for selection of an appropriate and accurate antifungal drug.[62] Therefore antifungal susceptibility testing has become an important aspect of patient management and resistance surveillance. The Clinical Laboratory Standards Institute (CLSI), formerly the National Committee on Clinical Laboratory Standards (NCCLS) has approved M27A3 for macrobroth and microbroth dilution and M44-A for disc diffusion susceptibility testing of yeast and yeast like fungi.[62]

CLSI standardized microbroth dilution methodology is the reference method for antifungal susceptibility testing of yeast and yeast like fungi.[62] In contrast to the macrobroth dilution method, it is easy and less labor intensive.[63] At present, there are two widely accepted standards for microbroth dilution methodology; the CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST).[61] Both the methods provide qualitatively and quantitatively similar MIC data for azole and echinocandin antifungal agents.[61] Availability of commercial systems based on the microbroth dilution methodology has reduced complexity and time required for antifungal susceptibility testing of *Candida* spp.[61] VITEK 2 (Biomerieux Inc., Hazelwood, MO, USA), is an example of fully automated commercial antifungal susceptibility testing system. This method is reliable and rapid and has good reproducibility when compared with CLSI reference broth microdilution method.[61]

Disc diffusion (figure 2) and E test are simple yet comparable with CLSI standardized broth dilution method.[61] In developed countries antifungal susceptibility testing is now increasingly used to supplement the treatment of fungal infections whereas, in developing countries where use CLSI standard broth method and sophisticated commercial kits is not always feasible, simple and user friendly methods like disc diffusion and E test can be easily incorporated.[61]

Most of research publications on antifungal resistance in *Candida* spp. are focused on triazoles. Azole group of antifungal agent impairs fungal cell synthesis of ergosterol by inhibiting the cytochrome p450 dependent enzyme sterol 14-α-demethylase.[56] This results in increased membrane permeability and inhibition of cell growth and reproduction.[56] These drugs are safe and effective for treatment of various clinical types of *Candida* infections.

Fluconazole is the most common triazole used for treatment and prophylaxis of candidiasis. [64] Its efficacy has been reported to be equivalent to amphotericin B in the treatment of candidemia. [65] As per the current guidelines on treatment of mycotic infections, fluconazole is the first drug of choice in clinically stable patients who are not on azole prophylaxis and infected with fluconazole susceptible *Candida* spp. [27] It is a cost effective and most readily available antifungal drug with good bioavailability and water solubility but has narrow spectrum of activity.[66, 67] Fluconazole is

available in oral and intravenous formulation. Empirical use of fluconazole both for therapeutic and prophylactic purpose has increased resistance to this drug. NAC spp. like *C. glabrata* and *C. krusei* are intrinsically resistant to fluconazole.[27] *C. dubliniensis* is innately sensitive to fluconazole, but rapidly develop resistance during the course of treatment.[68] Increased resistance to this drug is also reported in *C. tropicalis*.[13] Secondary resistance to fluconazole can develop in *C. albicans* isolates.[27] Fluconazole prophylaxis is one of the major contributing factors responsible for shift in epidemiology of candidiasis from *C. albicans* to NAC spp.

Figure 2: Antifungal susceptibility testing of *Candida* spp by disc diffusion method on Mueller Hinton glucose methylene blue agar

As compared to fluconazole, itraconazole has wider antifungal spectrum against *Candida* spp. It has improved activity against *C. glabrata* but lacks reliable activity against *C. krusei*.[27] Now a day, it is mostly used in treatment of mucocutaneous candidiasis.[27]

Voriconazole, the 2nd generation synthetic triazole, is structurally related to fluconazole.[69] It has broad spectrum activity against *Candida* spp. As voriconazole inhibits 24-methylene dihydrolanosterol demethylation in yeast, it is active against fluconazole-resistant or less susceptible NAC spp. like *C. glabrata* and *C. krusei*.[66, 69] Therefore, it is a more potent azole with a wide antifungal activity compared to fluconazole. Voriconazole is an effective antifungal drug for DC. It is more active than amphotericin B against both azole susceptible and azole resistant species of *Candida*.[69] *C. rugosa*, an emerging pathogen from NAC spp. is reported to be resistant to voriconazole.[14]

Posaconazole is a lipophilc 2nd generation triazole, is structurally related to itraconazole.[66] But in contrast to itraconazole, it has fluorine substituent in place of chlorine and furan ring inplace of dioxolane ring.[70] *In vivo* and *in vitro* studies conducted by various researchers have demonstrated broad spectrum activity of this drug against various *Candida* spp.[70] It blocks ergosterol synthesis in fungi by inhibiting lanosterol 14α-demethylase. Posaconazole is active against fluconazole resistant *C. albicans* and NAC spp. like *C. guilliermondii*, *C. krusei*, *C. norvegenesis* and *C. inconspicua*.[70] It is available only as oral suspension and is currently not approved for treatment of DC; however, it is effective for OPC and in salvage therapy for DC.[70] Posaconazole cross-resistance with fluconazole, itraconazole, or both, has been shown in some *Candida* isolates.[70] This resistance is species specific.[70]

Amphotericin B, a polyene was the accepted treatment of systemic candidiasis prior to availability of fluconazole and itraconazole.[27] It binds to ergosterol, the most important sterol in fungal membrane and alters the cell permeability, finally leading to leakage of cellular contents and cell death.[71] It has broad spectrum activity against *Candida* spp (except few strains of *C. glabrata*, *C. krusei* and *C. lusitaniae*).[27] Amphotericin B is associated with side effects like nephrotoxicity and electrolyte disturbances. To overcome nephrotoxicity associated with amphotericin B, a variety of reformulated versions have been introduced.[27] Lipid formulation of amphotericin B is the best example of the reformulated versions. Currently, three lipid formulations are available; these include amphotericin B lipid complex (ABLC-Abelcet, Zeneus Pharma), amphotericin B colloidal dispersion (ABCD-Amphocil, Cambridge Laboratories, Europe and Amphotec, Intermune, USA) and a liposomal formulation (L-AmB-Ambisome, Gilead Sciences).[27, 64] Other reformulated versions are in various stages of clinical development, this also include preparations that can be administrated orally. Liposomal amphotericin B is one of the first-line drugs for treatment of DC.[64]

The Echinocandins are the most recent addition to antifungal arsenal.[66] They are amphilic lipopeptides, products of cyclopentamine, which is fermentative product of fungi *like Zalerion arboricola* and *Aspergillus nidulans var. echinulatus*.[66] All three compounds of echinocandins (caspofungin, micafungin and

anidulafungin) have fungicidal action (both *in vitro* and *in vivo*) against most *Candida* spp.[72] MIC of all three echinocandins compound are much lower compared to amphotericin B and fluconazole against most of *Candida* spp. except *C*. *parapsilosis* and *C*. *guilliermondii*. MIC values for these two isolates are similar to those of fluconazole and amphotericin B i.e. 0.004-0.015 mg/l.[72]

Use of echinocandins have significantly increased in many hospital and they are now considered first-line drugs for treatment of DC among patients who are critically ill, clinically unstable, or have a history of recent exposure or colonization with a *Candida* spp known to have reduced susceptibility to azole.[73] As per the current guidelines, all three echinocandins are equally effective for the treatment of DC. [27] As echinocandins have been recently approved, data on its resistance pattern is limited. However few available studies reported echinocandin resistance in *C. albicans* and NAC spp. like *C. glabrata* and *C. parapsilosis*.[73] In addition, there is no information regarding whether increased use of echinocandins has resulted in better outcomes (i.e. decreased mortality) for DC patients. More multicentric studies are needed to define the role of echinocandins in the antifungal armamentarium.

CONCLUSION

Various factors have been implicated in the increased occurrence of *Candida* infections, but the widespread use of certain medical practices such as increased use of intravenous catheters, total parenteral nutrition, broad spectrum antibiotics and advent of HIV/AIDS are significant. Although *Candida albicans* is the most common cause of candidiasis, the shift towards treatment resistant non albicans *Candida* (NAC) spp. is evident in recent years. NAC spp. once less isolated (*C. glabrata*, *C. krusei* and *C. tropicalis*) or not isolated (*C. rugosa*, *C. norvegenesis* and *C. inconspicua*) are increasingly reported.

As the epidemiology of candidiasis is not constant, the incidence rates, species distribution and even antifungal susceptibility profiles seems to be changing. The changing trends of candidiasis underscore the need of continuous monitoring and up to date knowledge for effective prophylaxis and treatment.

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