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CANDIDA DIAGNOSTIC PLATFORMS: ESSENTIAL IN EARLY MANAGEMENT OF *CANDIDA* INFECTIONS

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ABSTRACT
Invasive candidiasis (IC) is associated with high morbidity and mortality. Candida species have significant roles in invasive candidiasis and the emergence of clinical strains that are difficult to treat due to virulent and resistant properties. Expansion of fungal pathogen diversity necessitates the development and adoption of new methods for prompt diagnosis and management. The review aimed to highlight the relevance of different Candida diagnostic methods for prompt management of Candida infections. The conventional gold standard diagnostic phenotypic-based (culture) method is time-consuming, associated with low specificity and subjective interpretation. The specificity and sensitivity performances for candidaemia or deep-seated candidiasis of biochemical-based methods, including VITEK, API 20C AUX, and latex agglutination, have higher resolution than the culture. Nucleic acid-based polymerase chain reaction diagnostic techniques have been rapidly evolving. PCR will improve the diagnostic performance and patient outcome. The PCR technique uses different ribosomal DNA gene complexes, including D1/D2, ITS1/ITS4, or IGS1/IGS2, as helpful markers to delineate the main pathogenic fungal species belonging to different genera. Sensitive and specific diagnostic methods for Candida species are significant for clinical decision and effective clinical outcome.

Keywords: Invasive candidiasis; diagnosis; culture; PCR; biochemical methods

INTRODUCTION

Candida species are common pathogenic yeasts associated with and threatening invasive infections. The species cause diseases such as oral, oesophageal, vulvovaginal, intra-abdominal infections, and invasive candidiasis. The above Candida infections have a predictable annual incidence of ~2.3 million, ~134 million, ~650 000, and ~80 000 cases, respectively (Arastehfar et al., 2019). Candidiasis is a general term used to describe a fungal infection caused by any Candida genus member. It can be superficial, involving the mucosal membranes (e.g., oral and genital) and the skin. It is highly prevalent in both healthy immunocompetent and immunocompromised patients (Davidson et al., 2018; Zhou et al., 2018). Candida meningoencephalitis is a significant cause of neurological morbidity (Kontoyiannis 2019). The Candida infections of the skin, nails, oropharyngeal mucosa, oesophagus, and genital tract are merely occasional and do not extend to severe form (Bongomin et al., 2017a; Davidson et al., 2018). However, severe recurrent or persistent mucocutaneous candidiasis exists even in the absence of commonly associated predisposing factors, including immune defects, microbial imbalance, loss of skin and mucosal barrier malfunction. The condition is termed chronic mucocutaneous candidiasis (Bamba et al., 2018; Davidson et al., 2018).

Escalating invasive candidiasis, particularly in immunocompromised populations and other individuals at risk, is an emerging concern (Arvanitis et al., 2014). Diagnosis of invasive fungal infections is challenging because of uncommon presentation, cost, time consumption, limited and complicated methods, difficulty in species identification, and limited DNA testing availability (Marcelo & Farooq, 2018). The problem is further worsened because of the ~50% loss of sensitivity of the gold standard diagnostic method (culture). Other techniques used include biochemical methods (VITEK, API 20C AUX) and latex agglutination that shows non-reliable results. The techniques suffer limitations of sensitivity, specificity, expertise dependence, and high turn-around-time (TAT) (Figure 1). The development of new diagnostic tools for accurate and early identification of Candida species is necessary to reduce the susceptible populations' mortality rate. The biochemical methods' negative predictive value can be inadequate in very high-risk selected populations of candidaemia (Pappas et al., 2018).

The most well-known molecular technique is the polymerase chain reaction (PCR) because of its promising relevance in diagnosing many infectious agents. PCR-based diagnostic methods have provided fast, accurate and sensitive tools for the identification of many Candida species.The technique uses high-resolution markers ITS1/ITS4, D1/D2, or IGS1/IGS2 on rDNA

to delineate closely related fungal species (Gago, 2014). The DNA sequence variation in molecular markers provides fungal evolutionary distinction (Xu and Adamowicz, 2016). The review aimed to highlight the relevance, advantages and disadvantages of different methods used to detect Candida species in the context of sensitivity, specificity, and TAT for better patient management.

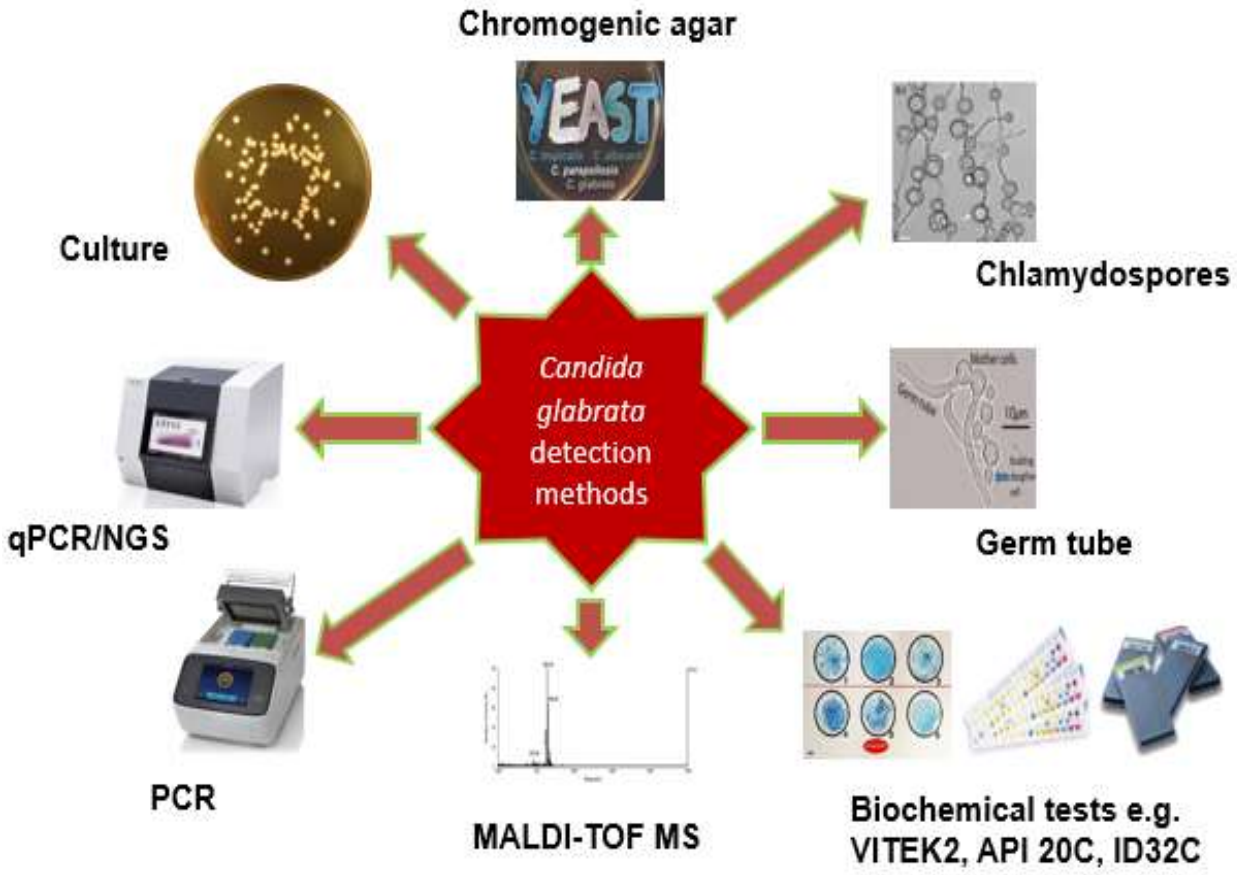

Figure 1: Current diagnostic methods for the detection of *Candida* species

Culture-based *Candida* detection methods

Culture method

The culture method is quite sensitive at detecting viable *Candida* even from a blood sample. According to Pfeiffer *et al.* (2011), the cultural method's sensitivity to detect *Candida* from a blood sample is one colony-forming unit (CFU) per mL. Blood cultures detect *Candida* species from infected patients only when a sample is collected during active candidaemia. According to Clancy and Nguyen (2018), the culture is only positive in ~40% of patients with candidemia, and infection is complicated by deep-seated infection. The infection persists after *Candida* has been cleared from the bloodstream. Across the spectrum of invasive candidiasis patients tested, blood cultures' sensitivity is ~50%, with high turnaround (TAT) gross limitations.

In some cases, the blood culture only turns positive during the late course of the disease. Fidler et al. (2018) viewed that culture is limited based on *Candida* species' phenotypic similarities and high TAT of detection to the identification. It may take 2 to 8 days, delays diagnosis and initiation of appropriate antifungal treatment with serious mortality consequences.

Fungal selective media such as SDA with gentamicin, brain heart infusion (BHI), and inhibitory mould agar (IMA) have increased sensitivity and shorten TAT. They have a superior advantage over the high TAT conventional fungal culture media (Hong et al., 2017). However, selective media clinical relevance in identifying patients with candidiasis or deep-seated candidiasis is primarily unknown. Several factors contribute to the reduced sensitivity of culture in proving candidaemia.The factors include:

(i) Fewer numbers (low burden of yeast cells of ~10 to 25 cells per 10 ml of blood) during candidaemia
(ii) Sample collected from sterile sitesis only ~50% sensitive, likely reflecting small sample volumes,
(iii) Use of inappropriate media that may inhibit fungal growth or the presence of antimycotics in the blood sample (Arvanitis et al., 2014; Vanhe et al., 2010).
(iv) Furthermore, deep tissue sample collection for cultures requires invasive procedures that may likely pose a risk. Thus, contraindicated in patients at-risk for *Candida* infections with serious untoward consequences (Babu et al., 2010).

Primary diagnostic methods, including microscopy, microbiological culture, and histopathology, could not meet physicians'urgency in clinical decisions (Bongomin et al., 2017b). Hence, developing more sensitive molecular-based techniques to meet the urgent demand for making a clinical decision.

Chromogenic agar candida media

Chromogenic agar candida media is another commercial method of *Candida* detection and speciation. It is produced by CHROMagarTM Candida medium and CandiSelectÒ 4 agar (BioRad). These agar media contain chromogenic substrates that react with a secreted enzyme from specific *Candida* species to produce specific colour reactions as an end-point character on the media for differentiation (Phoompoung & Chayakulkeeree, 2016; Yahaya et al., 2014). Chromogenic media diagnostic values are only high among *C. albicans, C. tropicalis,* and *C. krusei.* Sometimes inaccuracies may develop due to enzyme variation among the species (Phoompoung and Chayakulkeeree, 2016). Chromogenic agar candida media's main limitation is the inconsistent production of specific colours associated with specific *Candida* species. This limitation is observed in *C. glabrata* which may appear white, pink, or purple, and *C. krusei,* which is originally pinkish but may appear white. The variability of end-point colouris significant in missing the actual causative agent (Rodrigues et al., 2014; Sasso et al., 2017). *Candida* colony appearance on CHROMagar candida is not discriminatory as many non-*albicans Candida* species produce similar light pink colonies. The organisms include *C. krusei, C. kefyr, C. guilliermondii, C. auris, C. haemulonii, C. glabrata,* and *C. parapsilosis* (Kumar et al., 2017).

Chlamydospore formation test

This method is also known as the cornmeal agar technique or Dalmau plate technique. It is a method used to identify chlamydospore producing *Candida* species. These species include *C. albicans, C. dubliniensis,* and *C. tropicalis* (few strains). The cornmeal agar (CMA) is a nutritionally deficient media that supports chlamydospore development in the presence of tween 80 (Deorukhkar, 2018; Yahaya et al., 2015). The distinctive features of *Candida* species commonly encountered include the formation of different shapes of pseudohyphae, filamentous growth, and chlamydospores (Melhem et al., 2013). Pseudohyphae with plenty of spherical blastoconidia at the constriction point and thick-walled large round terminal single chlamydospores are recognized features of *C. albicans.* Isolates exhibiting pseudohyphae with bunches of terminal chlamydospores are regarded as *C. dubliniensis*. *Candida parapsilosis* strains appear like a Christmas tree with branched pseudohyphae that gradually becomes smaller at the distal end. The *C. tropicalis* isolates are identified by displaying long branching with abundant pseudohyphae. It also indicates a narrow sterile apex and ellipsoid blastoconidia. *Candida glabrata* shows clusters of small, budding blastospores without pseudohyphae formation (Saha et al., 2009). Bharathi (2018) reported modifications that improved the conventional CMA method's sensitivity by adding 5% milk. The milk promotes *C. albicans* growth and chlamydospore formation. This method's limitation includes the high TAT of approximately 48 h, laborious, requires specific skills to speciate the isolates, and depends on primary culture before CMA analysis (Bharathi, 2018).

Conventional methods (culture and biochemical tests) are being replaced progressively by molecular approaches, particularly PCR-based techniques and proteomics in reference centres (Merseguel et al., 2015). Although these conventional techniques are used in some health centres, they suffer gross limitations of loss of sensitivity and high TAT. The technique cannot detect non-culturable species and false identification of rare and cryptic species (Costa et al., 2016; Merseguel et al., 2015).

Non-culture-based methods

Germ tube test

The germ tube test is a simple screening method for detecting *C. albicans* from other *Candida* species first described by Taschdjian in 1960. The germ tube is a small and continuous non-septate elongation of the mother cell that initiates the *C. albicans* filamentation. The germ tube's parallel walls do not have a constriction at their point of origin of blastoconidium. It is considered a transitional morphological switch between the yeast and germinating hyphae (Souza et al., 2015). *Candida albicans* and *C. dubliniensis* use the switch as pathogenic properties to increase protein synthesis and ribonucleic acid (Seow et al., 2014). The distinct feature differentiates germ tube from pseudohyphae produced by other *Candida* species (Deorukhkar, 2018). Target *Candida* species are incubated at 37°C for 2-4 h in the presence of human serum or proteinaceous media like egg white, saliva, sheep serum. Others include fetal bovine serum, peptone water, or trypticase soya broth. The use of human serum is associated with inherent safety issue (Deorukhkar, 2018).

Candida dubliniensis is a closely related organism to *C. albicans* with many phenotypic similarities. Both species produce germ tubes, chlamydospores on cornmeal or Rice-Tween agars, and green colour on ChromAgar media. Pal's (sunflower seed), tobacco, and Niger seed agars have been proposed as methods to distinguish the two species. However,

none of these phenotypic methods alone is entirely reliable in separating them (Brandt and Lockhart, 2012). The sensitivity is also increased by using serum-free yeast extract peptone dextrose (YEPD) media at 39°C incubation. This yields more consistent results than the conventional methods compared to serum-induced germ tube test and chlamydospore test. This method's sensitivity allows the differentiation of *C. albicans* from *C. dubliniensis* (Jan et al., 2018).

Despite this advancement, the method is deficient because of emerging non-*albicans* resistant strains that do not produce germ tubes of medical relevance (Silva et al., 2012). This method's limitation is the dependence on a pure culture that takes a minimum of 24 h before the germ tube test. When it is negative, the decision requires the addition of approximately 48 h to check for chlamydospore formation using cornmeal agar (Mirhendi et al., 2011). It has high TAT, delays the clinical decision, and results in poor outcomes with severe consequences (Nabili et al., 2013). Moreover, Deorukhkar (2018) recent report indicated *Candida africana* as a germ tube producer, so the method is inadequate and needs further analysis for decision.

Biochemical methods

Phenotypic or biochemical assays are tests usedfor the identification of *Candida* species. The tests include enzyme immunoassays for circulating mannans and biochemical tests, and(1→3)-β-D-glucan are essential laboratory tools for diagnosing invasive candidiasis (Perlin et al., 2017). In contrast, mannan, β-D-glucan, and antimannan antibodies biomarkers of *Candida* species show variable results and disappointing performance (Pappas et al., 2018). Other methods consist of VITEK, API 20C AUX, latex agglutination, BD Phoenix, carbon source assimilation, and ID32C system (Delavy et al., 2019; Kordalewska et al., 2017). These techniques have shown low TAT within four hours and had long been validated for application in antifungal susceptibility testing (AFST) within 6-8 hours (She and Bender, 2019). These available commercial phenotypic methods (e.g.,VITEK2 and API 20C) for identifying *C. glabrata* from each member of the Nakaseomyces clade are helpful for preliminary identification. They are inadequate in identifying the group members (Norimatsu et al., 2017). However, the molecular method is necessary for confirmation (Dudiuk et al., 2017).

VITEK2 system plays a dual role in the identification and antifungal susceptibility testing (Melhem et al., 2013). Despite these valuable attributes, they fail to identify new emerging strains such as *Candida auris*, *Candida hominis, Candida duobushaemulonii,* and *Candida haemulonii*. Because of the lack of entries in FDA-approved libraries, they are frequently misidentifying these organisms. In a study conducted in India in search of *C. auris*, the VITEK2 system indicated less specificity by detecting more *C. haemulonii* yeasts than *C. auris* (Oberoi et al., 2012). VITEK2 system is used for AFST application with higher MIC values observed in amphotericin B. It causes inappropriate prescription of antifungal therapy with severe consequences on patients' health (Kim et al., 2009). It is significant to note that the range of reported MIC values of the VITEK 2 system is limited, making the interpretation of intermediate isolates difficult. The condition is mainly observed with caspofungin, as the lower limit of reporting (0.25 mg/L) covers both susceptible and intermediate isolates of *C. glabrata*, according to published CLSI breakpoints (Cretella et al., 2016).

API 20C is also a phenotypic method used for the detection of *Candida* species. The API 20C system uses 20-cupule plastic strips containing dehydrated substrates of carbohydrates. The fungal suspension added to the dehydrated substrate causes a change of colour, read after incubation. The method is simple to conduct; however, sometimes difficult to interpret the colour intensity differences. It is time-consuming; it takes up to 72 h to observe the colour change (Ramani et al., 2014).

Moreover, the method is inadequate to address the detection of emerging strains, particularly *C. auris* and its related species (Lee et al., 2011). The API 20C system misdiagnosed *C. auris* to *Rhodotorula glutinis* with the tendency of severe health consequences. Chatterjee et al. (2015) viewed that API 20C regularly misidentifies *C. auris* as *C. haemulonii*or *C. famata*.It indicatesthe under-reporting of the actual occurrenceof *C. auris*fungemia in the affected health facilities. Mandviwala et al. (2010) also reported misidentification of one isolate from 32 test isolates by API 20C profile as *C. dubliniensis*, instead of *C. glabrata*as confirmed by DNA sequence analysis.

The ID 32C system is another phenotypic method made up of single-use disposable plastic strips. It contains 32 wells of substrates for 29 assimilation tests for carbohydrates, organic acids, and amino acids. As it occurs to other phenotypic systems, it is easy to operate but too demanding, and high TAT attributes are associated with it. It is also associated with the misidentification of the actual target organism. Merseguel et al. (2015) reported seven inconclusive results using the ID 32C system, which were later confirmed as different *Candida* species using the PCR method. The identified species include one *C. lusitaniae*, three *C. intermedia*, two *C. haemulonii,* and one *C. duobushaemulonii.*

Molecular-based *Candida* detection methods

The polymerase chain reaction is referred to as the "gold standard" for molecular analysis due to its resolution effect on the differentiation of microorganisms. PCR techniques show promising benefits in speed, economy, and resolution power (Fidler et al., 2018). It provides early and direct detection of microorganisms (e.g., *Candida* species) from the blood. It allows for microbiological data documentation of invasive candidiasis (Arnaud et al., 2018).

However, PCR involves multiple analytical steps, ranging from sample pre-treatment, target gene amplification, and amplicon detection. It runs in a confined laboratory setting with expensive instruments, making it very difficult for low resource laboratories to adopt for routine diagnosis (Craw and Balachandran, 2012). Other drawbacks include the requirement of highly skilled personnel and high turnaround time. According to the view of Chang et al. (2012), the approach of PCR requires costly thermal cycling instrumentation, considerable expertise, and a sizeable space in routine diagnostic laboratories. Thus, its use is limited to highly sophisticated reference and research facilities. These shortfalls

resulted in the development of improved molecular-based techniques, which are rapid, sensitive, specific, and cost-effective (Craw and Balachandran, 2012). Moreover, the PCR technique relies on the necessity of using only agarose gel as a detection method of the amplified DNA, which adds to its operational costs (Abdullahi et al., 2015). A study conducted by Wang et al. (2016) indicated that physiological differences between individual rDNA repeats exist despite their identical DNA sequence.

The relevance of the ITS gene for phylogenetic study

The internal transcribed spacer (ITS) region is located between the 18S and 28S ribosomal RNA (rRNA) genes of fungal species. It separated into the ITS1 region, located between the 18S small subunit (SSU) and 5.8S rRNA genes, and the ITS2 region, located between 5.8S and 28S large subunit (LSU) rRNA genes (Khodadadi et al., 2017). The amplicon sizes vary according to the target ITS1 region based on specific species of interest (Korabecna, 2007; Romanelli et al., 2014). Genetic tools (e.g., sequence) provides vast information on many species' biology, particularly emerging *Candida* species and their drug resistance profile (Dadar et al., 2018). The ITS region of ribosomal DNA (rDNA) serves as a genetic marker for sequence analysis. It appears to be the most consistent strategy for the accurate and rapid molecular identification of fungal pathogens that infect humans (Sachse et al., 2003; Uemura et al., 2008).

The use of rDNA for sequence analysis bridges phenotypic methods' limitations because of its sound accuracy, high-resolution effect, and rapidly distinguishes strictly related species (Wagner et al., 2018). Moreover, sequence variability in the ITS region also shows relevance in the taxonomy, phylogeny, and population dynamics. These generally improve the discrimination of various *Candida* species and strains (Chen et al., 2000; Iwen et al., 2002) (Figure 2).

The internal transcribed spacer of rDNA is potentially useful as a molecular biomarker for evaluating genetic constitution among significant clinical isolates of *Candida* species. It is also helpful to characterize inter and intraspecies, as recently reported by Merseguel et al. (2015). The ITS region's resolution capacity resulted in the separation of species;*ab initio* regarded as *C. glabrata* from *C. glabrata* complex. The complex is derived from Nakaseomyces clade and is human pathogenic species, including *C. nivariences*, *C. bracarensis*, and *C. glabrata* based on the ITS gene's sequence differences (Mirhendi et al., 2011). Even though they can be phenotypically separated, however, there is a need to utilize the molecular methodology to prove the diagnosis reported by Angoulvant et al. (2016). There is variability and antifungals resistance among the species (Arvanitis et al., 2014; Dudiuk et al., 2017; Kasper et al., 2015). The ITS1 and ITS2 usually are species-specific, unlike the intercalary 5.8S gene that is highly conserved (Khodadadi et al., 2017). It is often vital to rely on the most variable regions (ITS1 and ITS2) for sequencing analysis (Figure 2).

The ITS has become the most prevalent genetic marker for fungal identification and molecular taxonomy. There are four main reasons for its good popularity: universal primers' availability and the genome's multicopy structures. The improved amplification efficiency and sensitivity of detection of the suspected agent, i.e., the availability of numerous copies of the ribosomal operon, makes primer design and PCR amplification of the ITS region easy. The ITS region'srelatively limited length allows natural amplification and sequencing. Its good resolution power leads to species discrimination in most fungal taxa due to the high evolutionary rates of particularly ITS 2 gene (Fidler et al., 2018; Tafoya-Ramírez et al., 2018). Another relevance of the ITS gene is that it has several available sequences much more abundant than any available gene. The conservation of sequences in the proximate genes, together with multicopy structure, allows for successful amplification even from low-DNA-yield extraction (Bengtsson-Palme et al., 2013).

The significant advantages of these variable genes are allowing different molecular techniques to identify targeted fungal pathogens.It includesconventional PCR amplification followed by direct sequencing of target DNA or fragment size analysis, pyrosequencing, and microarray analysis.Additionally, thedevelopmentof several hybridization-based platforms that combine PCR amplification of the ITS1/ITS2 target regions and specific molecular probes for subsequent hybridization (Walker, 2009). The findings of Xu and Adamowicz (2016) showed the potential roles of fungal barcoding in mycology studies. Currently, there is much fungal diversity in different ecological niches, yet described. The ITS sequences, mainly the ITS1 region, is often adequate to distinguish many analyzed macrofungi. The result promises well for old herbarium specimens where long, high-quality DNA is often difficult to extract due to DNA degradation and breakage. Despite a few limitations, the application of the ITS region of ribosomal DNA (rDNA) for sequence analysis is the most reliable strategy for the accurate molecular identification of fungal pathogens that infect humans (Merseguel et al., 2015).

The relevance of the D1/D2 region

Domains 1 and 2 (D1/D2) are a variable region within the large 28S ribosomal DNA subunit commonly used to identify yeasts and yeasts-related organisms (Sumerta and Kanti, 2018).The region has a divergence fragment size of the approximate length of around ± 600 bp (Sumerta and Kanti, 2018). The region is informative owing to its variable nature and can result in genus-specific and species-specific identifications (Backx et al., 2014). Medically relevant fungal species have high taxonomic diversity. Different genetic loci, including the D1/D2 domain of the 28S rRNA large subunit (LSU), are commonly applied in *Candida* species detection (Irinyi et al., 2016). Currently, the ITS and D1/D2 approach in conventional PCR and sequencing served as the gold standard to detect and confirm the strains of *C. auris* with 100% specificity and sensitivity and shorter TAT (Milena et al., 2017; Osei Sekyere, 2018). Tsay et al. (2018) also viewed that the D1/D2 domain of the 28S rDNA and ITS are the most reliable regions for molecular detection of *C. auris* (Tsay et al., 2018).

According to Koricha et al. (2019), a total of 182 yeast strains belonging to 16 genera and 27 species were successfully identified based on the sequence analysis of the D1/D2 region. Compared with ITS1, the D1/D2 LSU region exhibits much

lower length heterogeneity and intra-strain sequence divergence in fungi (Hanafy et al., 2020). Figueiredo-Carvalho et al. (2016) reported that the genomic sequence analysis using both ITS and D1/D2 regions of the tested clinical isolate showed 100% similarity with *C. nivariensis* rather than *C. glabrata* as earlier perceived. Koricha et al. (2019) reported that the D1/D2 variable domain helped differentiate the yeasts isolated from the different edible tree samples using PCR analysis.The region is simple to align; it is hyper-variable and universally available in a database for all known yeast species. It is also used earlier than the ITS region. Mohd Tap et al. (2018) viewed that sequencing of the PCR amplicons of ITS and D1/D2 domain in LSU regions is a reliable method for identifying rare *Candida* species. Gade et al. (2017) viewed the D1/D2 as the hyper-variable region capable of identifying mucormycetes from formalin-fixed and fresh tissues. Findings of many reviewed studies showed that ITS region is more reliable than D1/D2 as frequently used to detect the emerging *C. auris*.

The relevance of the IGS1/IGS2 regions

The intergenic spacer (IGS) of the rDNA is found between tandem repeats of transcription rDNA units. Each repeat unit of fungal groups has a separately transcribed coding gene for 5S rRNA. The separation of IGS into two smaller regions (IGS1 and IGS2) by the 5S rDNA gene makes PCR amplification easier (Fischl, 2015; Gago et al., 2014) (Figure 2). The IGS region is one of the most suitable markers in the rDNA array. It is rapidly evolving to detect many fungal species' inter-and intraspecies diversity,including studies of *Saccharomyces cerevisiae, Cryptococcus neoformans* and *Trichosporon* species (Gago et al., 2014; Sutar et al., 2004). The IGS region is present in multiple copies per genome and is organized as tandem arrays with the highest variability (Gálvez et al., 2020).

Kasahara et al. (2014) reported the use of the IGS1 region as a target. According to Niu et al. (2012), the IGS region is the least conserved in the entire length of the rDNA repeat clusters from a wide variety of organisms. They reported 5S rDNA-IGS2 regions conserved property within *Meloidogyne* species. It exhibited intense discrimination of *M. enterolobii*from other *Meloidogyne* species. According to the findings of Xia et al. (2008) the IGS1 region showed higher sensitivity and specificity than those of the D1/D2 and ITS regions in the identification of phylogenetically closely related *Trichosporon* species. It was able to discriminate between the closely related *Trichosporon asahii* and *Trichosporon mucoides*. The view was in accordance with Marcelo and Farooq (2018) report that IGS1 is perhaps the best marker able to delineate all known *Trichosporon* species. The higher variability helps identify and distinguish the closely related *Fusarium* species (Gálvez et al., 2020). Cebeci et al. (2017) viewed that the IGS with *AluI* fingerprinting method was applied to study *Meyerozyma guilliermondii* isolates. It is based on the experience that ITS DNA sequencing could not provide adequate differentiation for its identification. Despite these vast advantages, IGS sequence data for some organisms, including Sclerotiniaceae, is not readily available in the data bank for phylogenetic analysis and identification (Hirschhäuser and Fröhlich, 2007).

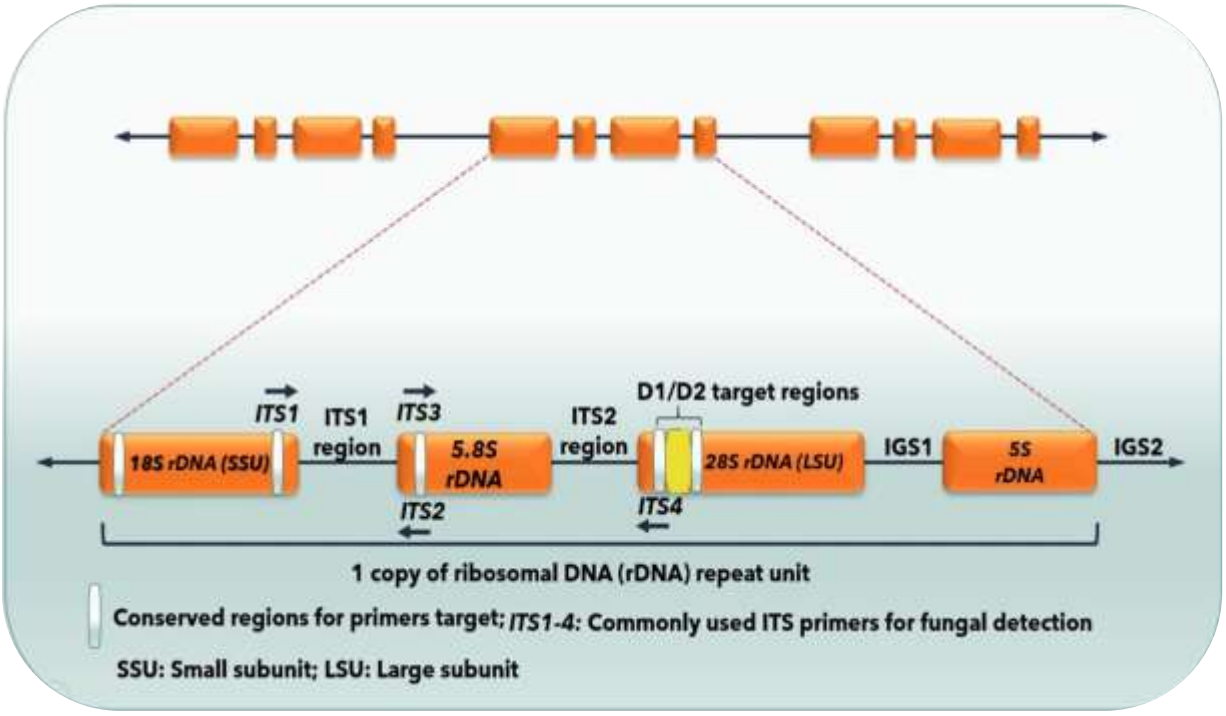

Figure 2: Ribosomal DNA repeat unit showingITS1/2, D1/D2, and IGS1/2 regions used for fungal detection

Other applied detection methods

MALDI-TOF MS (spectrometry)

Matrix associated laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has recently been counted as a suitable, accurate, rapid, and high throughput technology in the identification of different microorganisms (bacteria and fungi) at the species level (Delavy et al., 2019). MALDI-TOF MS is used with increased frequency for rapid species identification in clinical settings and for the taxonomic evaluation of fungi (Nathan et al., 2018). It works based on fingerprinting detection of specific protein released from the microbial cells. It thus generates spectral peaks for comparison to standardized data in the database (Spanu et al., 2012). The reliability of MALDI-TOF MS has attracted the attention of many researchers for rapid identification of *Candida* species. Despite their strong cell wall, the technique shows robustness compared to culture and other phenotypic methods (Arendrup and Patterson, 2017; Delavy et al., 2019).

MALDI-TOF MS provides an alternative approach to detect species-specific metabolites between organisms. It has short TAT by detecting targets within a few hours with total accuracy, similar to DNA barcoding for many taxonomic groups (Xu and Adamowicz, 2016). MALDI imaging currently has a dimensional resolution between 5–50 µm, allowing analysis of a single cell within a given tissue (Prosser et al., 2014). Another significant relevance of MALDI-TOF MS is its application for direct detection of antifungal resistance in clinical isolates without conventional antifungal susceptibility testing (AFST) methods. The AFST methods include disk diffusion, broth microdilution, E-test, or even the gold standard agar dilution methods with reasonable accuracy (Delavy et al., 2019).

Despite the MALDI-TOF MSadvantages, its limitations include inaccurate data deposits in the reference database for comparison. It is labour-intensive, and the method's outcome depends on pure fungal culture. Another drawback includes a missing protein with many different molecules, resulting in the target's misdiagnosis (Xu and Adamowicz, 2016). MALDI-TOF MS can correctly identify *C. auris* from the closely related species (Vatanshenassan et al., 2019). The authors reported correct identification (100%) of all 23 *C. auris* clinical isolates and the 10 strains from the centre for disease control and prevention (CDC).

Candida Magnetic resonance

The T2Candida® (T2 Biosystems, Lexington, MA, USA) is a miniaturized, fully automated, and culture-independent panel diagnostic approach based onmagnetic resonance (Lamoth et al., 2020). The T2Candida can directly detect and rapidly identifythe causative agent of candidemiafrom a patient whole blood sample (Chowdhary et al., 2016). It offers cell species-specific *Candida* rapid detection and identification within 3–5 h (McCarty and Pappas, 2016). The technique detects the five most common *Candida* species*C. albicans, C. tropicalis, C. parapsilosis, C. glabrata*and *C. krusei,*from a whole blood sample (Pappas et al., 2018; Zervou et al., 2017). It detectsvarious targets, including molecular targets (e.g., DNA); immunodiagnostics (e.g., proteins); and a broad range of hemostasis markers. The method works based on the principle that measures how water molecules react in the presence of magnetic fields (Bilir et al., 2015).

Accordingly, the whole blood is subjected to PCR amplification of *Candida* species, followed by hybridization of single-stranded sequences to nanoparticles that cause a T2 magnetic resonance signal alteration. T2 relaxation signal indicates the target organism's absence (Arvanitis et al., 2014; Duncan et al., 2016). The technologies have been approved by the Food and Drug Administration (FDA) of the United States of America (Lamoth et al., 2018; McCarty and Pappas, 2016). It enables more rapid TAT than PCR assays of approximately 12 h and blood cultures of approximately five days. It is critical for the early management of invasive candidiasis.

The performance of T2Candida helps in predicting the patients' outcomes and less expensive in various settings (Falces-Romero et al., 2018; Tang et al., 2019). Lamoth *et al.* (Lamoth et al., 2020) reported the performance of T2Candida for detecting intra-abdominal candidiasis (IAC) in 48 high-risk patients. T2Candida has 33% sensitivity and 93% specificity results. According to Iguchi et al. (105), the study on 89 clinical samples (*C. auris* culture-positive 46, negative 43) indicated the sensitivity of 0.89 and specificity of 0.98 at a 95% confidence interval. The overall sensitivity per assay of the T2Candida® was 91.1%, and the overall specificity was 99.4%. It identifies a bloodstream infection missed by blood cultures while retaining sensitivity during antifungal therapy (Kontoyiannis et al., 2019*).*

Future directions

Severe consequences of clinical outcomes due to IC necessitates the development and adoption of new reliable methods with very short TAT results. For example, the development of loop-mediated isothermal amplification (LAMP), which is rapid, simple, non-PCR machine-dependent, and operates at constant temperature (60-65°C), will be a future direction for easy management of IC patients. Moreover, the LAMP method may allow point-of-care testing (POCT) integration and higher sensitivity than conventional PCR and real-time quantitative PCR (qPCR). The speed of the method helps to detect IC early for better management of patients.

CONCLUSIONS

Antifungal resistance and virulence in *Candida* species are on the increase. They are emerging threats to patient management and clinical success. The problem is further deteriorating by the emergence of *C. auris* that surpasses all other *Candida* species as the most challenging pathogen to identify and treat. Diagnostic strategies with high sensitivity, specificity and short TAT could significantly shifts cenarios from the current state towards more reliable assays to better manage candidiasis patients.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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