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Exploring Molecular Methods for Candida species Identification and Characterization

Sushree Swagatika Subhadarsini[1,2], Monali Priyadarsini Mishra[1], Debasmita Dubey[3], Gopal Krishna Purohit[2]*

[1]School of Paramedics and Allied Health Sciences, Centurion University of Technology and Management, Bhubaneswar, India, [2]Heredity Biosciences LLP, Bhubaneswar, Odisha, [3]Medical Research Laboratory, IMS & Sum Hospital, Siksha O Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India. *Corresponding Author's Email: gopalpurohit2000@gmail.com

Abstract

Candida species (CS) are significant fungal pathogens responsible for a wide range of infections in humans, making their precise and timely identification crucial for effective treatment and disease management. Traditional identification methods, such as phenotypic assays and culture-based techniques, often suffer from limitations, including prolonged processing times, limited accuracy, and inadequate differentiation between species. As a result, molecular techniques have gained prominence for their ability to rapidly and accurately identify and characterize various Candida species. This review discusses the shortcomings of conventional methods and highlights the array of molecular strategies that have been developed, such as polymerase chain reaction (PCR) assays, DNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). These techniques leverage the unique genetic and proteomic profiles of Candida species to provide highly sensitive and specific identification, even distinguishing between closely related strains. Additionally, the review explores the use of molecular approaches in clinical diagnostics, epidemiological studies, and antifungal resistance monitoring, where they enable the rapid detection of Candida species from clinical samples, allowing for swift diagnosis and timely antifungal intervention. Furthermore, these techniques are instrumental in identifying clonal outbreaks and tracking transmission dynamics in healthcare settings. The review emphasizes the advantages of molecular methods—such as their precision, speed, and capacity to uncover cryptic species—while also calling for continued research to improve their efficacy and broader application in Candida species identification.

Keywords: Antifungal Resistance, Candida Species, Clinical Mycology, DNA Sequencing, MALDI-TOF MS, PCR.

Introduction

Candida species are significant as opportunistic fungal pathogens that can trigger various humans' infections. Precise identification and characterization of these species are essential for effective treatment, selecting the right antifungal agents, and understanding the spread and behaviour of candidiasis. While traditional identification methods, such as culture-based techniques and phenotypic assays, have been useful, they suffer from issues like slow processing times, limited accuracy, and difficulty in distinguishing between species. As a result, the adoption of molecular techniques has transformed this area of study. These modern methods offer a faster, more accurate way to identify and differentiate Candida species by analysing their genetic and proteomic profiles. This review discusses the advancements in molecular techniques that are enhancing the identification and characterization of Candida species, focusing on their implications for clinical practice and epidemiological studies (1). One of the key molecular techniques employed for Candida species identification is polymerase chain reaction (PCR). PCR assays targeting specific Candida genes or regions can rapidly amplify and detect Candida DNA, enabling sensitive and specific identification (2). Additionally, DNA sequencing technologies, such as Sanger sequencing and next-generation sequencing (NGS), have provided comprehensive genotypic analysis, allowing for precise species identification and the detection of genetic markers associated with antifungal resistance and virulence (3). Matrix-assisted laser desorption/ionization time-of-flight mass

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spectrometry (MALDI-TOF MS) has emerged as a powerful tool for rapid and accurate Candida species identification. MALDI-TOF MS analyses the protein profiles of microbial isolates and can differentiate Candida species based on unique mass spectra patterns (4). Molecular techniques, including multilocus sequence typing (MLST) and whole-genome sequencing (WGS), have greatly enhanced our insight into the epidemiology of Candida outbreaks. By examining the genetic profiles of Candida strains, these methods enable the identification of clonal outbreaks, facilitate the tracking of specific strain distributions, and help clarify the origins and pathways of transmission (5).

Overview: Candida Species

Candida species belong to the kingdom Fungi, phylum Ascomycota, class Saccharomycetes, family Saccharomycetaceae, and genus Candida. These unicellular yeasts exhibit a variety of shapes, typically ovoid or spherical (6). They have an incomplete sexual reproduction cycle. Commonly, Candida species are harmless residents in the human body, inhabiting areas like the respiratory system, gastrointestinal tract, vaginal mucosa, oral cavity, and skin in healthy people. Additionally, they are found in various external environments such as plants, water, and soil. These yeasts are versatile in their ability to break down proteins and carbohydrates, which serve as essential sources of carbon and nitrogen for their growth (7). The relationship between native species of Candida and human hosts is shaped by numerous elements, such as pathogenic, physiological, mechanical, and medically induced factors. This interaction enables Candida species to trigger various infections with a broad spectrum of clinical symptoms. These can vary from mild, superficial infections to severe, invasive diseases that can compromise several organs and potentially lead to the death of the host (8). Among the nearly 200 different Candida species, a small number of them are of particular clinical significance. The most prevalent ones include *Candida albicans* (9-11*), Candida tropicalis (*12*), Candida glabrata (*13, 14), *Candida krusei* (15), and *Candida parapsilosis* (16) (which further include *Candida orthopsilosis* and *Candida metapsilosis*). These species are responsible for more than 90% of invasive Candida infections (15). Additionally, there are emerging Candida species, such as *Candida guilliermondii* (15), *Candida dubliniensis (*17*)*, *Candida lusitaniae*, *Candida kefyr*, *Candida rugosa*, *Candida famata*, *Candida utilis*, *Candida lipolytica*, *Candida norvegensis*, and *Candida inconspicua*, which have clinical relevance and have been identified as causative agents of both superficial and systemic infections (18).

Importance of Accurate Candida Species Identification

Accurate identification of Candida species is crucial for several reasons, significantly impacting clinical outcomes, therapeutic decisions, and epidemiological insights into candidiasis. By precisely identifying Candida species, clinicians can tailor treatment strategies to the specific fungal pathogen, enhancing the efficacy of antifungal therapies. This level of specificity not only leads to better patient management but also aids in understanding the spread and behaviour of different Candida species within populations. Ultimately, accurate Candida species identification ensures that patients receive the most effective treatment, promoting faster recovery and minimizing the risk of complications associated with inappropriate or ineffective therapy.

Impact on Patient Management

Accurate species identification helps in determining the appropriate antifungal therapy for candidiasis. Different Candida species may exhibit varying susceptibilities to antifungal agents. For example, *Candida glabrata* and *Candida krusei* are known to be less susceptible to azole antifungals, while *Candida albicans* is generally more susceptible. Therefore, accurate identification allows tailored treatment approaches based on the specific Candida species involved (18).

Selection of Targeted Antifungal Therapy

Certain antifungal agents may have better efficacy against specific Candida species. For instance, echinocandins are considered the treatment of choice for invasive Candida infections caused by *Candida glabrata* or *Candida krusei*, while azoles are commonly used for *Candida albicans* infections. Accurate identification of the causative species enables the selection of the most appropriate antifungal therapy, optimizing treatment outcomes (18, 19).

Understanding the Epidemiology of Candidiasis

Accurate species identification plays a vital role in tracking the epidemiology of candidiasis. It helps identify the prevalence of different Candida species in various geographic regions and healthcare settings, as well as detect trends in antifungal resistance. This knowledge is essential for implementing appropriate infection control measures and guiding public health policies to combat the spread of resistant *Candida species* (18, 19).

Limitations of Traditional Methods for Candida Species Identification

Traditional methods for Candida species identification, such as phenotypic assays and culture-based techniques, have several limitations that can impact the accuracy and efficiency of identification (20). These limitations highlight the need for more advanced molecular methods in Candida species identification.

Phenotypic Assays

Phenotypic assays rely on the observation of various characteristics, such as colony morphology, biochemical reactions, and growth at different temperatures or on specific media. However, these methods may lack specificity and accuracy, leading to misidentification or difficulty in distinguishing closely related Candida species (21). Furthermore, phenotypic assays may require extended incubation periods, resulting in delays in obtaining conclusive identification.

Culture-based Techniques

Culture-based techniques involve the growth of Candida isolates on specific agar media followed by morphological examination. While these methods have been traditionally used, they have limitations, including the requirement for skilled laboratory personnel, time-consuming procedures, and the potential for contamination or overgrowth by other microorganisms (22).

Challenges in Species Differentiation

Some Candida species exhibit similar morphological and biochemical characteristics, making their differentiation challenging using conventional methods alone. For example, distinguishing *Candida dubliniensis* from *Candida albicans* or accurately identifying emerging species with clinical significance, such as *Candida auris*, can be difficult (23, 24).

Accuracy in Detecting Mixed Infections

Traditional methods may struggle to detect mixed Candida infections accurately, where more than one species coexist. These infections can be particularly problematic as different species may respond differently to treatment and have varying degrees of pathogenicity (13).

Overview of Molecular Methods for Candida Species Identification

Molecular methods have revolutionized the field of Candida species identification by providing rapid, accurate, and reliable results. These techniques utilize the genetic information of Candida species to enable precise identification and characterization Table 1. Below, Table 1 provides an overview of commonly used molecular methods for identifying Candida species, highlighting their respective advantages and limitations.

Table 1: Comparison of Molecular Methods for Candida Species Identification

Method	Principle	Target Gene(s)	Advantages	Limitations
PCR-RFLP	PCR amplification followed by restriction fragment length polymorphism	ITS region, D1/D2 region of 28S rRNA gene, or other genomic regions	Rapid, cost-effective, high discriminatory power	Requires post-PCR manipulation, may lack species specificity
Real-time PCR	PCR amplification with real-time detection of fluorescent signal	ITS region, other conserved regions	High sensitivity and specificity, quantitative analysis possible	Expensive instrumentation, susceptibility to PCR inhibitors
Multiplex PCR	Simultaneous amplification of multiple target sequences	ITS region, other conserved regions	High throughput, simultaneous detection of multiple species	Primers design can be challenging, increased risk of

				nonspecific amplification
DNA Sequencing	Determination of nucleotide sequence of target DNA fragment	ITS region, D1/D2 region of 28S rRNA gene, other genomic regions	High accuracy, species-level identification	Time-consuming, expensive, requires specialized equipment and expertise
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry	Protein profiling	Rapid, high-throughput, accurate	Database limitations, may require extensive sample preparation
DNA Microarray	Hybridization of DNA probes to target sequences on a microarray	Species-specific probes	High throughput, simultaneous detection of multiple species	Expensive, limited availability of commercial arrays, interpretation of results may be complex

Polymerase Chain Reaction (PCR) Assays

PCR assays are widely employed for the detection and identification of Candida species. These methods amplify specific regions of the Candida genome, such as the internal transcribed spacer (ITS) region, to generate DNA fragments that can be analysed. PCR-based assays offer high sensitivity, specificity, and speed, allowing for the rapid identification of Candida species from various clinical specimens (25).

DNA Sequencing Techniques

DNA sequencing methods, including Sanger sequencing and next-generation sequencing (NGS), have significantly advanced Candida species identification. Sanger sequencing involves determining the nucleotide sequence of target genes, such as the ITS region, to identify Candida species based on genetic variations. NGS technologies allow for the simultaneous sequencing of multiple Candida isolates, providing comprehensive genomic information and facilitating the detection of novel or emerging species (26).

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) MALDI-TOF MS has emerged as a powerful tool for the rapid identification of Candida species. It relies on the detection of unique protein profiles in Candida isolates, allowing for quick and accurate species identification. MALDI-TOF MS has demonstrated high sensitivity and specificity and can be integrated into routine laboratory workflows for efficient Candida species identification (27).

Multilocus Sequence Typing (MLST)

MLST involves sequencing multiple conserved genetic loci in Candida isolates to determine their genetic diversity and relatedness. By comparing the sequences of specific genes among different Candida species, MLST can provide insights into their phylogenetic relationships and population structures. This method is particularly useful for epidemiological studies and tracking the spread of Candida infections (28).

Biochemical Assay Techniques for *Candida* Identification

Biochemical assay techniques for *Candida* species identification, such as sugar assimilation, chromogenic media, and enzymatic activity assays, have been foundational in microbiology labs. These methods allow clinicians to differentiate *Candida* species by studying their metabolic and biochemical profiles, though they come with several limitations, especially in clinical settings where rapid and accurate diagnosis is crucial.

Sugar Assimilation and Fermentation Tests

One of the most commonly used biochemical approaches; these tests analyze how various *Candida* species metabolize different sugars. For example, *Candida albicans* ferments glucose, maltose, and sucrose, while *Candida glabrata* is more limited, fermenting only glucose and trehalose (29). These distinctions form the basis for identifying species. Systems like API 20C AUX and VITEK 2 have automated these processes, making them more efficient. However, despite automation, these tests often take 24 to 72 hours to generate results, which can delay the treatment

of critical infections. Moreover, they struggle to differentiate closely related species like *Candida albicans* and *Candida dubliniensis*, leading to potential misdiagnosis, particularly when species have overlapping sugar assimilation profiles (30).

Chromogenic Media

Chromogenic media, like CHROMagar Candida, provide a more rapid option, yielding results in 24 to 48 hours by producing visually distinct colony colors. For instance, *Candida albicans* appears as green colonies, while *Candida krusei* produces pink colonies (31). This simplicity makes it a popular choice, especially for laboratories seeking faster presumptive identification. However, chromogenic media have limitations, particularly with less common species. Some species, such as *Candida parapsilosis*, form white or cream-colored colonies, making it difficult to distinguish between them (32). This technique also lacks the ability to identify mixed infections, which could complicate treatment plans if one species is dominant while others are overlooked.

Enzymatic Activity Assays

These assays focus on the production of enzymes, such as phospholipase or proteinase, which are associated with the pathogenicity of different *Candida* species. For instance, *Candida albicans* and *Candida tropicalis* produce high levels of phospholipase, while *Candida glabrata* does not. While enzyme production is valuable for understanding virulence, it's not always reliable for species identification due to variability in enzyme expression, which can depend on environmental conditions (33). Additionally, these tests require extended incubation times, further delaying the diagnostic process.

Limitations of Biochemical Assays

Sensitivity and Specificity Issues

Many biochemical assays cannot differentiate between species with similar metabolic profiles, like *Candida albicans* and *Candida dubliniensis* (34). Misidentification of species can lead to incorrect treatment choices, which is particularly problematic given that these species may respond differently to antifungal drugs.

Prolonged Turnaround Time

Biochemical methods are relatively slow, often taking days to deliver results. In cases of invasive candidiasis, where every hour counts, delays in diagnosis can result in worse patient outcomes. Rapid diagnosis is crucial for initiating appropriate antifungal therapy and improving patient survival rates (35).

Inability to Detect Cryptic or Rare Species

Biochemical methods may fail to identify emerging or rare pathogens like *Candida auris*, which have become significant due to their antifungal resistance (36). Cryptic species, which may appear morphologically similar to more common species, require more advanced methods, like molecular techniques, for accurate identification (37).

Limited Use in Mixed Infections

These assays may not effectively detect mixed infections, where multiple *Candida* species are involved. Identifying only the dominant species could lead to incomplete treatment strategies, as the undetected species may contribute to the infection's resistance profile or pathogenicity (38).

Advantages of Molecular Methods over Traditional Approaches

Molecular methods for Candida species identification offer several advantages over traditional approaches, providing more accurate, rapid, and reliable results, as outlined in Table 2. Some key benefits of molecular methods include:

Increased Accuracy and Specificity

Molecular methods target specific genetic regions or sequences, enabling precise identification of Candida species. These methods can differentiate closely related species that may have similar phenotypic characteristics, reducing the chances of misidentification (2, 39). By detecting species-specific genetic markers, molecular methods enhance the accuracy and specificity of Candida species identification.

Rapid Turnaround Time

Molecular methods provide significantly faster results compared to traditional techniques. Techniques such as PCR assays and MALDI-TOF MS can deliver species identification within hours, allowing for timely decision-making regarding patient management and appropriate antifungal therapy selection (25, 27, 40). This rapid turnaround time is crucial in managing invasive Candida infections, where timely treatment is critical for patient outcomes.

Enhanced Sensitivity

Molecular methods exhibit higher sensitivity for Candida detection, even at low fungal loads. PCR assays, for example, can amplify and detect Candida DNA even when the organism is present in small quantities, increasing the chances of accurate

detection in clinical specimens (27). This enhanced sensitivity enables the detection of Candida species that may have otherwise been missed by conventional culture-based methods.

Detection of Mixed Infections

Molecular methods are valuable in detecting mixed Candida infections, where multiple species coexist. By amplifying and analysing specific genetic targets, these methods can identify and differentiate multiple Candida species present in a single clinical sample, aiding in tailored treatment approaches (41-44). This capability is particularly beneficial in complex clinical scenarios and immunocompromised patients.

Potential for Simultaneous Detection of Resistance Markers

Molecular methods can also be employed to detect antifungal resistance markers in Candida species. This enables the identification of drug-resistant strains and helps guide appropriate antifungal therapy selection (45-48). Combining species identification with resistance detection in a single assay provides a comprehensive understanding of the clinical isolate's characteristics.

This Table 2 compares molecular technologies (e.g., PCR, MALDI-TOF) and traditional identification techniques (biochemical assays, culture-based methods) for *Candida* species identification. It evaluates key factors such as sensitivity, specificity, turnaround time, cost, and clinical feasibility, highlighting the strengths and limitations of each method in diagnostic settings.

Table 2: Comparative Analysis of Molecular Technologies vs. Traditional Identification Techniques for *Candida* Species

Criteria	**Molecular Technologies (e.g., PCR, MALDI-TOF, DNA Sequencing)**	**Traditional Identification Techniques (Biochemical Assays, Culture-Based)**
Sensitivity	- High sensitivity - Can differentiate closely related species and cryptic species - Detects mixed infections and low fungal loads (36)	- Moderate to low sensitivity - Difficult to distinguish cryptic or rare species - Requires sufficient fungal growth for accurate detection (49)
Specificity	- Very high specificity - Targets species-specific genetic or proteomic markers - Reduces chances of misidentification (50)	- Variable specificity - May misidentify closely related species due to overlapping metabolic profiles - Limited for non-*albicans Candida* species (51)
Time	- Rapid results (hours) - PCR, MALDI-TOF can provide same-day results - Suitable for urgent clinical decisions (52)	- Slow turnaround time (days to weeks) - Requires fungal growth, metabolic tests, and manual interpretation - Time-consuming for critical care cases (35)
Cost	- Higher upfront cost due to equipment and reagents - Reduced long-term cost with automation - Economical for high-throughput laboratories (53)	- Lower upfront costs - Consumables (media, reagents) are generally less expensive - High costs in the long term due to repeated testing and delays (54)
Feasibility in Clinical Settings	- Highly feasible in well-equipped, modern laboratories - Requires trained personnel and specialized equipment - Limited access in resource-limited settings (55)	- Feasible in most clinical labs - Can be performed without specialized equipment - Easily accessible in low-resource settings (15)
Detection of Antifungal Resistance	- Can detect antifungal resistance markers (e.g., *ERG11* mutations) - Real-time PCR can guide targeted therapy (56)	- Limited in resistance detection - Requires separate tests for susceptibility, which adds to time and cost (57)

Detection of Mixed Infections	- Can detect mixed species in a single assay (e.g., multiplex PCR) - Crucial for co-infections that influence treatment outcomes (58)	- Limited ability to detect mixed infections - Dominant species may mask the presence of others (59)
Application in Epidemiological Studies	- Useful for outbreak detection and transmission tracking - Can perform strain-level typing (36)	- Less effective for strain typing - Limited utility for tracking transmission and genetic diversity (60)

Addressing Technical Challenges in PCR for *Candida* Species Identification

While PCR offers significant advantages in sensitivity and specificity for the identification of *Candida* species, several technical challenges can complicate its application in clinical laboratories. These include the potential for false positives and false negatives, difficulties with DNA extraction from clinical samples, contamination risks, and PCR inhibition. Addressing these issues is crucial for ensuring the accuracy and reliability of PCR-based methods in routine diagnostics.

False Positives and False Negatives

PCR assays, although highly sensitive, can be susceptible to both false positives and false negatives. False positives often occur due to contamination with extraneous DNA, particularly in high-throughput laboratories handling multiple samples. This is a critical concern in *Candida* identification, as even minute traces of fungal DNA can lead to misidentification. Conversely, false negatives may arise if the fungal DNA concentration in the sample is too low or if the target DNA sequence is mutated or degraded (61, 62). To mitigate false positives, stringent contamination control measures are essential. These include the use of separate areas for DNA extraction, reagent preparation, and PCR amplification, as well as the use of negative controls in every assay batch (63). For false negatives, sample quality is critical; ensuring adequate fungal load through optimized sample collection and storage, coupled with the use of highly sensitive primers that target conserved genetic regions of *Candida* species, can significantly reduce the risk of undetected infections (35).

DNA Extraction from Clinical Samples

One of the most significant challenges in PCR-based *Candida* identification is the extraction of high-quality DNA from clinical samples, such as blood, tissues, or sterile body fluids. Clinical samples often contain inhibitors that can interfere with PCR reactions, such as heme in blood or complex proteins in tissues. These inhibitors can reduce PCR efficiency and lead to false negative results (64). To address these issues, protocols for DNA extraction must be optimized to ensure the purity and concentration of fungal DNA, free from inhibitors. Commercially available DNA extraction kits specifically designed for fungal pathogens, as well as additional purification steps like ethanol precipitation or silica column-based clean-ups, are commonly used to overcome these challenges. Additionally, real-time PCR techniques can include internal amplification controls to monitor for PCR inhibition and validate the results of each assay (65).

Contamination Control and PCR Inhibition

Cross-contamination is a well-known risk in molecular laboratories, especially in high-sensitivity assays like PCR. Given that *Candida* species are common environmental contaminants and can colonize laboratory spaces, rigorous contamination control practices are crucial. Use of aerosol-resistant pipette tips, frequent decontamination of workspaces with DNA-degrading agents, and inclusion of "no template" controls can reduce contamination risks (66). PCR inhibition is another technical challenge that can arise from the presence of substances that interfere with DNA polymerase activity. Common inhibitors in clinical samples include haemoglobin, bile salts, and other organic compounds. To address PCR inhibition, it is essential to use extraction protocols that minimize these contaminants. Additionally, incorporating PCR enhancers, such as bovine serum albumin (BSA), into the reaction mixture can help overcome inhibition, improving the reliability of results (67).

Practical Considerations for Clinical Laboratories

For PCR-based assays to be adopted in clinical laboratories, it is vital to ensure their practicality in routine diagnostics. This includes standardizing protocols for sample processing, DNA extraction,

and amplification to ensure consistency and reproducibility. The use of real-time PCR assays with automated systems can also streamline workflow, reducing the manual handling of samples and decreasing the risk of human error. Finally, proper training of laboratory personnel in handling molecular techniques and contamination prevention is essential to maintain the reliability of PCR assays for *Candida* species identification.

Application of Molecular Methods in Epidemiological Studies

Molecular methods have proven to be valuable tools in epidemiological studies focused on understanding the transmission, spread, and genetic diversity of Candida species. The application of these methods provides important insights into the dynamics of Candida infections and aids in the development of effective control strategies.

Strain Typing and Genotyping

Molecular methods such as multilocus sequence typing (MLST), amplified fragment length polymorphism (AFLP), and pulsed-field gel electrophoresis (PFGE) enable the characterization and comparison of Candida strains at a genetic level. These techniques allow for the identification of clonal clusters, genetic relatedness, and the tracking of specific strains within and between healthcare facilities (68, 69).

Understanding Antifungal Resistance Mechanisms

Molecular methods are instrumental in studying the genetic mechanisms underlying antifungal resistance in Candida species. By detecting and characterizing resistance-associated genes or mutations, these methods provide valuable insights into the emergence and spread of resistance (15, 23, 70). This knowledge is essential for monitoring trends in resistance and optimizing antifungal treatment strategies.

Population Structure and Evolutionary Analysis

Molecular methods allow for the study of Candida population structure and evolutionary relationships. By analysing the genetic diversity and phylogenetic relationships among Candida isolates, researchers can gain insights into the global distribution, transmission patterns, and evolutionary history of Candida species (28, 71). This information is crucial for understanding the epidemiology of Candida infections and designing targeted control measures.

Prominent Candida Species Involved in Infections

Several Candida species have been identified as significant pathogens in various clinical infections. Understanding the prevalence and clinical significance of these species is essential for accurate diagnosis and effective management of Candida infections. Among the prominent Candida species involved in infections, *Candida albicans* remains the most common and clinically relevant species (72). It accounts for a significant proportion of both superficial and invasive Candida infections, including candidemia, oral thrush, and vaginal candidiasis. *Candida tropicalis* is another important species associated with invasive candidiasis, particularly in immune-compromised patients (72). It has been found to exhibit intrinsic resistance to certain antifungal drugs, highlighting the need for accurate identification and appropriate treatment selection. *Candida glabrata* has emerged as a significant pathogen, especially in nosocomial infections and among immune-compromised individuals (16). It is known for its ability to develop resistance to commonly used antifungal agents, posing challenges in treatment. *Candida krusei*, although less prevalent than other species, is noteworthy due to its inherent resistance to fluconazole, a commonly prescribed antifungal drug (73). This species is often associated with infections in patients with prior exposure to antifungal agents. *Candida parapsilosis*, consisting of three distinct subgroups (*Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis*), is increasingly recognized as a cause of healthcare-associated infections, particularly in neonates and patients with indwelling medical devices (74).

Clinical Relevance and Role of Candida Species in Various Types of Infections

Candida species play a significant role in a wide range of infections, ranging from superficial to invasive and systemic infections. Understanding their clinical relevance in different types of infections is crucial for appropriate diagnosis and management. Here is an overview of their roles in various infections:

Superficial Candida Infections

Candida species, particularly *Candida albicans*, are frequently associated with superficial infections

such as oral thrush (oral cavity), vulvovaginal candidiasis (vaginal mucosa), and diaper dermatitis (skin). These infections primarily affect mucosal surfaces and are commonly observed in otherwise healthy individuals (75).

Invasive Candidiasis

Candida species, including *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, and *Candida parapsilosis*, can cause invasive candidiasis, which involves the invasion of deeper tissues and organs. This includes candidemia (bloodstream infection), disseminated candidiasis, and deep-seated organ infections (1, 76). Invasive candidiasis typically occurs in immune-compromised individuals, such as those with compromised immune systems, critically ill patients, or those undergoing invasive medical procedures.

Candida-Associated Urinary Tract Infections (UTIs)

Candida species can also colonize and infect the urinary tract, leading to UTIs. Candida albicans is the most common species associated with urinary tract infections, although other species like *Candida glabrata* and *Candida tropicalis* can also be involved. Candida UTIs are more prevalent in patients with indwelling urinary catheters or underlying urinary tract abnormalities.

Gastrointestinal Candidiasis

Candida species, especially *Candida albicans*, can cause infections in the gastrointestinal tract, leading to conditions such as esophagitis, gastritis, and enteritis. Gastrointestinal candidiasis is commonly observed in individuals with weakened immune systems, such as HIV/AIDS patients or those undergoing chemotherapy.

Candida-Associated Skin and Nail Infections

Candida species can cause infections of the skin and nails, including cutaneous candidiasis and onychomycosis. These infections commonly occur in warm and moist areas of the body, such as skin folds or between the toes, and are frequently associated with factors like excessive moisture, poor hygiene, or compromised skin integrity.

Therapeutic Importance of Distinguishing Various *Candida* Species

The accurate identification of *Candida* species is a cornerstone of effective treatment, especially in the face of increasingly resistant strains. The diverse antifungal susceptibilities of *Candida* species, particularly between *Candida albicans* and the non-*albicans Candida* species, can significantly impact treatment outcomes. When *Candida albicans* is properly identified, treatment is relatively straightforward because it typically responds well to fluconazole, a commonly used antifungal. However, non-*albicans Candida* species like *Candida glabrata* and *Candida krusei* complicate things. *Candida glabrata* is known for its reduced susceptibility to azoles, including fluconazole, and it can develop resistance rapidly, making it crucial to use more potent antifungals, such as echinocandins or amphotericin B, right from the start (77). *Candida krusei*, on the other hand, is intrinsically resistant to fluconazole, so relying on traditional treatment approaches without accurate species identification can lead to treatment failure, which can be devastating in cases of invasive candidiasis (78). The emergence of *Candida auris* has taken these challenges to another level. This species, resistant to multiple antifungal classes, has caused outbreaks worldwide, especially in hospital settings where it survives well on surfaces and spreads between patients. The fact that *C. auris* is resistant to azoles, echinocandins, and polyenes makes it a nightmare in terms of treatment options, often requiring combination therapies and aggressive infection control measures (79). Traditional biochemical methods, while useful, have their limits. They often struggle with closely related species and cryptic ones, which can be harder to detect but still responsible for recurring or persistent infections. For instance, species like *Candida dubliniensis* and *Candida parapsilosis* may be missed or misidentified using older methods, leading to inappropriate treatments that don't target the actual pathogen (80). Molecular techniques, such as PCR and DNA sequencing, offer much more precision. These methods can accurately differentiate between species that share similar biochemical characteristics but have very different antifungal susceptibility profiles. This higher level of specificity is essential not just for acute treatment but also for managing recurrent infections, where the persistence of a resistant or less common species might otherwise go undetected.

Future Directions and Potential Advancements in Molecular Methods for Candida species Identification and Characterization

Molecular methods have revolutionized the field of Candida species identification and

characterization, providing rapid, accurate, and sensitive techniques. As technology continues to advance, there are several exciting future directions and potential advancements in molecular methods for Candida species identification.

Next-Generation Sequencing (NGS)

NGS technologies, such as whole-genome sequencing (WGS), hold great promise for Candida species identification and characterization. WGS allows for comprehensive genomic analysis, including the identification of genetic variations, virulence factors, and antifungal resistance genes, providing a deeper understanding of Candida species' pathogenicity and epidemiology (69).

Metagenomics

Metagenomic approaches enable the analysis of microbial communities in various environments, including human microbiota. Applying metagenomic sequencing to clinical samples can provide valuable insights into the composition and dynamics of Candida species populations, helping understand their role in health and disease (70).

Point-of-Care Testing (POCT)

Development of rapid and portable molecular diagnostic devices for Candida species identification holds significant potential for point-of-care testing. These devices could enable timely and accurate diagnosis in resource-limited settings, facilitating prompt initiation of appropriate antifungal therapy (41).

Multi-Omics Integration

Integrating multiple omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, can provide a comprehensive understanding of Candida species biology and pathogenesis. This multi-omics approach can reveal novel biomarkers, therapeutic targets, and mechanisms of antifungal resistance (81, 82).

Bioinformatics and Data Analysis

Advances in bioinformatics tools and data analysis methods are crucial for handling the vast amounts of genomic and clinical data generated by molecular methods. Developing robust and user-friendly bioinformatics pipelines will enhance data interpretation, facilitate comparative genomics, and improve our understanding of Candida species biology and evolution (83). These advancements hold the potential to revolutionize the field of Candida species identification and characterization, leading to improved diagnostics, personalized treatment strategies, and better management of Candida infections.

Discussion

The diagnosis and identification of *Candida* species (CS) have indeed come a long way, especially with the evolution of molecular techniques. Traditional methods, like phenotypic assays and culture-based techniques, have served as the backbone of fungal diagnostics for a long time. However, as the review points out, these methods are fraught with challenges—particularly in terms of time and accuracy. Waiting days or even weeks for cultures to grow just doesn't fit the urgency required in clinical settings, especially when dealing with invasive candidiasis, where quick, targeted intervention is critical. The shift towards molecular techniques—such as PCR, DNA sequencing, and MALDI-TOF MS—has been a game-changer. These tools leverage the genetic and proteomic signatures unique to each *Candida* species, providing a level of precision that traditional methods struggle to match. For example, PCR-based tests allow clinicians to zero in on specific gene regions tied to certain *Candida* species, offering a more nuanced understanding of the infection at hand. DNA sequencing takes these even further, unravelling genetic variations between species. Meanwhile, MALDI-TOF MS is another fascinating option. It bypasses lengthy culture times by directly analysing proteins in a sample, making it a much faster route to species identification. One major benefit of these molecular approaches is speed. Instead of waiting for cultures to grow, these tests can often provide results within hours, allowing healthcare providers to jumpstart appropriate treatments far sooner. This can be life-saving in critical care scenarios, where delays in diagnosis can lead to worse patient outcomes. It's also vital in distinguishing between closely related species, like *Candida albicans* and *Candida dubliniensis*. These two may look nearly identical under traditional methods, but molecular techniques can reveal their differences, which is crucial because they may respond to antifungal treatments differently or carry varying pathogenic risks. Moreover, these techniques aren't just limited to diagnosing and treating individual cases. In a broader context, they are invaluable for epidemiological research and infection control. By identifying the specific strains causing outbreaks in hospitals or clinics,

healthcare teams can better track the spread of infections and implement containment measures more effectively. This is especially important with the rise of antifungal resistance, particularly concerning multidrug-resistant species like *Candida auris*. Being able to detect resistance patterns early can guide treatment decisions and help avoid the use of ineffective therapies. That said, molecular techniques do come with their own set of challenges. The cost and complexity of implementing these methods—particularly in resource-limited areas—can be a major barrier. They require specialized equipment and trained personnel, which may not always be available. Additionally, there's still a need for traditional culture methods in certain situations, particularly when antifungal susceptibility testing is needed. So, while molecular techniques are a fantastic leap forward, they don't fully replace older methods yet. Instead, integrating both old and new approaches into a comprehensive diagnostic workflow is often the best path forward.

Conclusion

In summary, the shift from traditional to molecular techniques represents a major breakthrough in the identification and characterization of Candida species. Molecular methods, such as PCR, DNA sequencing, and MALDI-TOF MS, provide substantial benefits in terms of speed, accuracy, and specificity over phenotypic assays and culture-based methods. These advanced molecular tools not only improve the precision of diagnosing and treating fungal infections but also play a critical role in monitoring epidemiology and antifungal resistance. With the increasing incidence of Candida infections, particularly among immune-compromised patients, the refinement and development of these techniques are essential. Future research should aim to enhance the sensitivity and specificity of these molecular methods, broaden their availability in diverse healthcare environments globally, and incorporate emerging technological innovations to stay ahead in combating fungal pathogens. The continued advancement of molecular methodologies will significantly strengthen our ability to manage Candida-related diseases, leading to improved patient outcomes and more effective control of these infections.

Abbreviations

CS: Candida Species, PCR: Polymerase Chain Reaction, DNA: Deoxyribonucleic Acid, MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry, ITS: Internal, Transcribed Spacer, NGS: Next Generation Sequencing.

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Author Contributions

Sushree Swagatika Subhadarsini led the manuscript development, conducting a thorough literature review, synthesizing information on molecular techniques for Candida species identification, and drafting the initial manuscript. Monali Priyadarshini Mishra supported material organization and collection, compiling relevant studies to ensure the review's depth and accuracy. Gopal Krishna Purohit oversaw the final revisions, ensuring scientific accuracy, narrative coherence, and alignment with journal standards, significantly enhancing the manuscript's overall quality.

Conflict of Interest

The authors declare no competing interests.

Ethics Approval

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References

1. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016 Feb 15;62(4):e1-50.
2. Magalhaes J, Correia MJ, Silva RM, Esteves AC, Alves A, Duarte AS. Molecular Techniques and Target Selection for the Identification of Candida spp. in

Oral Samples. Appl Sci. 2022;12(18):9204. https://doi.org/10.3390/app12189204
3. García-Salazar E, Acosta-Altamirano G, Betancourt-Cisneros P, Reyes-Montes MDR, Rosas-De-Paz E, Duarte-Escalante E, Sánchez-Conejo AR, Ocharan Hernández E, Frías-De-León MG. Detection and Molecular Identification of Eight Candida Species in Clinical Samples by Simplex PCR. Microorganisms. 2022 Feb 5;10(2):374.
4. Chen XF, Hou X, Xiao M, Zhang L, Cheng JW, Zhou ML, Huang JJ, Zhang JJ, Xu YC, Hsueh PR. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Analysis for the Identification of Pathogenic Microorganisms: A Review. Microorganisms. 2021;9(7):1536.
5. Alkhars N, Al Jallad N, Wu TT, Xiao J. Multilocus sequence typing of Candida albicans oral isolates reveals high genetic relatedness of mother-child dyads in early life. PLoS One. 2024 Jan 17;19(1):e0290938.
6. Shoff CJ, Perfect JR. Uncommon yeasts and molds causing human disease. Saccharomyces cerevisiae. Chapters and Articles, Encyclopedia of Mycology.
7. Wikipedia contributors. Candida albicans. In Wikipedia, The Free Encyclopedia. 2024 June 9. https://en.wikipedia.org/w/index.php?title=Candida_albicans&oldid=1225855299
8. d'Enfert C, Kaune AK, Alaban LR, Chakraborty S, Cole N, Delavy M, Kosmala D, Marsaux B, Fróis-Martins R, Morelli M, Rosati D, Valentine M, Xie Z, Emritloll Y, Warn PA, Bequet F, Bougnoux ME, Bornes S, Gresnigt MS, Hube B, Jacobsen ID, Legrand M, Leibundgut-Landmann S, Manichanh C, Munro CA, Netea MG, Queiroz K, Roget K, Thomas V, Thoral C, Van den Abbeele P, Walker AW, Brown AJP. The impact of the Fungus-Host-Microbiota interplay upon Candida albicans infections: current knowledge and new perspectives. FEMS Microbiol Rev. 2021;45(3). doi:10.1093/femsre/fuaa060.
9. Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, Škrlec I. Candida albicans-The Virulence Factors and Clinical Manifestations of Infection. J Fungi (Basel). 2021 Jan 22;7(2):79.
10. Mir MA, Rasool U, Aisha S, Alshehri B, Hamadani SS. Chapter 1 - Human pathogenic microbes (bacterial and fungal) and associated diseases. In: Human pathogenic microbes (bacterial and fungal) and associated diseases. Developments in Microbiology. 2022: 1-30. doi:10.1093/femsre/fuaa060.
11. Dick CF, Dos-Santos AL, Meyer-Fernandes JR. Inorganic phosphate uptake in unicellular eukaryotes. Biochim Biophys Acta. 2014;1840(7):2123-2127.
12. Wikipedia contributors. Candida tropicalis. In Wikipedia, The Free Encyclopedia. 2024 June 9. https://en.wikipedia.org/w/index.php?title=Candida_tropicalis&oldid=1210422538
13. Fidel PL Jr, Vazquez JA, Sobel JD. Candida glabrata: Review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. Clin Microbiol Rev. 1999;12(1):80-96.
14. Hassan Y, Chew SY, Than LTL. Candida glabrata: Pathogenicity and Resistance Mechanisms for Adaptation and Survival. J Fungi. 2021;7(8):667.
15. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Nagy E, Dobiasova S, Rinaldi M, Barton R, Veselov A; Global Antifungal Surveillance Group. Candida krusei, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J Clin Microbiol. 2008 Feb;46(2):515-21.
16. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev. 2012;36(2):288-305.
17. Pristov KE, Ghannoum MA. Resistance of Candida to azoles and echinocandins worldwide. Clin Microbiol Infect. 2019 Jul;25(7):792-798.
18. Dalyan Cilo B. Species Distribution and Antifungal Susceptibilities of Candida Species Isolated From Blood Culture. Cureus. 2023 Apr 27;15(4):e38183.
19. Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O. ESCMID guideline for the diagnosis and management of Candida diseases 2012: diagnostic procedures. Clin Microbiol Infect. 2013;19 (Supplement 3):12-15.
20. Lorenzo-Villegas DL, Gohil NV, Lamo P, Gurajala S, Bagiu IC, Vulcanescu DD, Horhat FG, Sorop VB, Diaconu M, Sorop MI, Oprisoni A, Horhat RM, Susan M, MohanaSundaram A. Innovative Biosensing Approaches for Swift Identification of Candida Species, Intrusive Pathogenic Organisms. Life (Basel). 2023;13(10):2099.
21. Kumar G, Shankar H, Bisht D, Sharma P, Singhal N, Katoch VM, Joshi B. A simple and rapid method of sample preparation from culture filtrate of M. tuberculosis for two-dimensional gel electrophoresis. Braz J Microbiol. 2010 Jun;41(2):295-9. https://doi.org/10.1590/S1517-83822010000200005
22. Vidotto V, Picelli A, Carlot M, Nadal E, Barbui AM, Mian P. Phenotypic and molecular methods for the identification of Candida famata: a comprehensive survey. Mycopathologia. 2016;181(7-8):551-558.
23. Jeffery-Smith A, Taori SK, Schelenz S, Jeffery K, Johnson EM, Borman A, Candida auris Incident Management Team, Manuel R, Brown CS. Candida auris: a review of the literature. Clinical Microbiology Reviews. 2018 Jan;31(1):10-128.
24. Borman AM, Linton CJ, Miles SJ, Campbell CK, Johnson EM. Ultra-rapid preparation of total genomic DNA from isolates of yeast and mould using Whatman FTA filter paper technology—A reusable DNA archiving system. Med Mycol. 2002;40(4):397-400.
25. Tsai MH, Lin LC, Hsu JF, Lai MY, Huang HR, Chiang MC, Lu JJ. Rapid identification of invasive fungal species using sensitive universal primers-based PCR and restriction endonuclease digestions coupled with high-resolution melting analysis. J Microbiol Immunol Infect. 2019;52(5):728-735.
26. Nilsson RH, Ryberg M, Abarenkov K, Sjökvist E, Kristiansson E. The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. FEMS Microbiol Lett. 2009 Jul;296(1):97-101. https://doi.org/10.1111/j.1574-6968.2009.01618.x
27. Lau AF, Drake SK, Calhoun LB, Henderson CM, Zelazny AM, Huggett JF. Development of a clinically comprehensive database and a simple procedure for

identification of molds from solid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2013;51(3):828-834.
28. Tavanti A, Davidson AD, Gow NA, Maiden MC, Odds FC. Candida orthopsilosis and Candida metapsilosis spp. nov. to replace Candida parapsilosis groups II and III. J Clin Microbiol. 2005;43(1):284-292.
29. Van Ende M, Wijnants S, Van Dijck P. Sugar Sensing and Signaling in Candida albicans and Candida glabrata. Front Microbiol. 2019 Jan 30;10:99.
30. Jabra-Rizk MA, Baqui AA, Kelley JI, Falkler WA Jr, Merz WG, Meiller TF. Identification of Candida dubliniensis in a prospective study of patients in the United States. J Clin Microbiol. 1999 Feb;37(2):321-6.
31. Nadeem SG, Hakim ST, Kazmi SU. Use of CHROMagar Candida for the presumptive identification of Candida species directly from clinical specimens in resource-limited settings. Libyan J Med. 2010 Feb 9;5(1).
32. Daef E, Moharram A, Eldin SS, Elsherbiny N, Mohammed M. Evaluation of chromogenic media and seminested PCR in the identification of Candida species. Brazilian Journal of Microbiology. 2014;45:255-62.
33. Turner SA, Butler G. The Candida pathogenic species complex. Cold Spring Harbor perspectives in medicine. 2014 Sep 1;4(9):a019778.
34. Bliss JM, Sullivan MA, Malone J, Haidaris CG. Differentiation of Candida albicans and Candida dubliniensis by Using Recombinant Human Antibody Single-Chain Variable Fragments Specific for Hyphae. J Clin Microbiol. 2003;41(3):1152-1160.
35. Khot PD, Fredricks DN. PCR-based diagnosis of human fungal infections. Expert Rev Anti Infect Ther. 2009 Dec;7(10):1201-21.
36. Tavanti A, Davidson AD, Johnson EM, Maiden MC, Shaw DJ, Gow NA, Odds FC. Multilocus sequence typing for differentiation of strains of Candida tropicalis. J Clin Microbiol. 2005 Nov;43(11):5593-600.
37. Arendrup MC, Patterson TF. Multidrug-resistant Candida: epidemiology, molecular mechanisms, and treatment. The Journal of infectious diseases. 2017 Aug 15;216(suppl_3):S445-51.
38. White TJ, Bruns T, Lee S, Taylor JW, Innis MA, Gelfand DH, Sninsky JJ. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: A Guide to Methods and Applications. San Diego: Academic Press; 1990:315-322.
39. Muñoz P, Bouza E, Cuenca-Estrella M, Eiros JM, Pérez MJ, Sánchez-Somolinos M, Rincón C, Hortal J, Peláez T. Saccharomyces cerevisiae fungemia: An emerging infectious disease. Clin Infect Dis. 2005;40(11):1625–1634. https://doi.org/10.1086/429916
40. Marinach-Patrice C, Fekkar A, Atanasova R, Gomes J, Djamdjian L, Brossas JY, Meyer I, Buffet P, Snounou G, Datry A, Hennequin C, Golmard JL, Mazier D. Rapid species diagnosis for invasive candidiasis using mass spectrometry. PLoS One. 2010 Jan 25;5(1):e8862.
41. Arvanitis M, Anagnostou T, Fuchs BB, Caliendo AM, Mylonakis E. Molecular and nonmolecular diagnostic methods for invasive fungal infections. Clin Microbiol Rev. 2014 Jul;27(3):490-526.
42. Camp I, Spettel K, Willinger B. Molecular Methods for the Diagnosis of Invasive Candidiasis. J Fungi (Basel). 2020 Jul 6;6(3):101. doi: 10.3390/jof6030101.
43. Fang W, Wu J, Cheng M, Zhu X, Du M, Chen C, Liao W, Zhi K, Pan W. Diagnosis of invasive fungal infections: challenges and recent developments. Journal of Biomedical Science. 2023;30:42. https://doi.org/10.1186/s12929-023-00926-2
44. Kidd SE, Chen SC-A, Meyer W, Halliday CL. A New Age in Molecular Diagnostics for Invasive Fungal Disease: Are We Ready? Front. Microbiol. 2020;10:2903. doi: 10.3389/fmicb.2019.02903
45. Fisher MC, Alastruey-Izquierdo A, Berman J, Bicanic T, Bignell EM, Bowyer P, Bromley M, Brüggemann R, Garber G, Cornely OA, Gurr SJ, Harrison TS, Kuijper E, Rhodes J, Sheppard DC, Warris A, White PL, Xu J, Zwaan B, Verweij PE. Tackling the emerging threat of antifungal resistance to human health. Nat Rev Microbiol. 2022;20:557–571. https://doi.org/10.1038/s41579-022-00720-1
46. Alastruey-Izquierdo A, Martín-Galiano AJ. The challenges of the genome-based identification of antifungal resistance in the clinical routine. Front. Microbiol. 2023;14:1134755. doi: 10.3389/fmicb.2023.1134755.
47. Ning Y, Xiao M, Perlin DS, Zhao Y, Lu M, Li Y, Luo Z, Dai R, Li S, Xu J, Liu L, He H, Liu Y, Li F, Guo Y, Chen Z, Xu Y, Sun T, Zhang L. Decreased echinocandin susceptibility in Candida parapsilosis causing candidemia and emergence of a pan-echinocandin resistant case in China. Emerg Microbes Infect. 2023 Dec;12(1):2153086.
48. Czajka KM, Venkataraman K, Brabant-Kirwan D, Santi SA, Verschoor C, Appanna VD, Singh R, Saunders DP, Tharmalingam S. Molecular Mechanisms Associated with Antifungal Resistance in Pathogenic Candida Species. Cells. 2023;12(22):2655.
49. Otašević S, Momčilović S, Stojanović NM, Skvarč M, Rajković K, Arsić-Arsenijević V. Non-culture based assays for the detection of fungal pathogens. J Mycol Med. 2018 Jun;28(2):236-248.
50. Song N, Zhou X, Li D, Li X, Liu W. A proteomic landscape of Candida albicans in the stepwise evolution to fluconazole resistance. Antimicrob Agents Chemother. 2022 Apr 19;66(4). doi: 10.1128/aac.02105-21.
51. Al Mosaid A, Sullivan DJ, Coleman DC. Differentiation of Candida dubliniensis from Candida albicans on Pal's agar. J Clin Microbiol. 2003 Oct;41(10):4787-9.
52. Arastehfar A, Fang W, Pan W, Liao W, Yan L, Boekhout T. Identification of nine cryptic species of Candida albicans, C. glabrata, and C. parapsilosis complexes using one-step multiplex PCR. BMC Infect Dis. 2018 Sep 25;18(1):480.
53. Köhler JR, Hube B, Puccia R, Casadevall A, Perfect JR. Fungi that infect humans. Microbiology spectrum. 2017 Jun 30;5(3):10-128.
54. Sankari SL, Mahalakshmi K, Naveen Kumar V. Chromogenic medium versus PCR-RFLP in the speciation of Candida: a comparative study. BMC Res Notes. 2019 Oct 22;12(1):681.
55. Daraskevicius J, Petraitis V, Davainis L, Zucenka A. The feasibility of ibrexafungerp for the treatment of

fungal infections in patients with hematological malignancies. J Fungi (Basel). 2022 Apr 23;8(5):440.
56. Mohamed AO, Mohamed SM, Hussain MA, Ahmed FI. Detection of antifungal drug-resistant and ERG11 gene mutations among clinical isolates of Candida species isolated from Khartoum, Sudan. F1000Res. 2020 Aug 26;9:1050.
57. Alastruey-Izquierdo A, Melhem MS, Bonfietti LX, Rodriguez-Tudela JL. Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology. Rev Inst Med Trop Sao Paulo. 2015 Sep;57 (Suppl 19):57-64.
58. Carvalho-Pereira J, Fernandes F, Araújo R, Springer J, Loeffler J, Buitrago MJ, Pais C, Sampaio P. Multiplex PCR Based Strategy for Detection of Fungal Pathogen DNA in Patients with Suspected Invasive Fungal Infections. J Fungi (Basel). 2020 Nov 23;6(4):308.
59. McManus BA, Coleman DC. Molecular epidemiology, phylogeny and evolution of Candida albicans. Infect Genet Evol. 2014;21:166-178.
60. Wojciechowska-Koszko I, Kwiatkowski P, Roszkowska P, Krasnodębksa-Szponder B, Sławiński M, Gabrych A, Giedrys-Kalemba S, Dołęgowska B, Kowalczyk E, Sienkiewicz M. Genetic diversity of Candida spp. isolates colonizing twins and their family members. Pathogens. 2022 Dec 13;11(12):1532.
61. Alanio A, Bretagne S. Difficulties with molecular diagnostic tests for mould and yeast infections: where do we stand? Clin Microbiol Infect. 2014; 20(Supplement 6): 36-41. https://doi.org/10.1111/1469-0691.12617
62. Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. J Clin Microbiol. 2011 Feb;49(2):665-70.
63. Xie M, Shao J, Wan Z, Yan T, Zhu S, Li S, Yu J. Detection of Candida DNA in peritoneal fluids by PCR assay optimizing the diagnosis and treatment for intra-abdominal candidiasis in high-risk ICU patients: A prospective cohort study. Front Microbiol. 2023;13:1070688.
64. Carvalho S, Costa-De-Oliveira M, Martins ML, Pina-Vaz C, Rodrigues AG, Ludovico P, Rodrigues F. Multiplex PCR identification of eight clinically relevant Candida species. Med Mycol. 2007;45(7):619–627. https://doi.org/10.1080/13693780701501787
65. Smith M. Validating real-time polymerase chain reaction (PCR) assays. Encyclopedia of Virology. 2021:35–44. doi: 10.1016/B978-0-12-814515-9.00053-9.
66. Arrieta-Aguirre I, Menéndez-Manjón P, Carrano G, Diez A, Fernandez-de-Larrinoa Í, Moragues MD. Molecular identification of fungal species through multiplex-qPCR to determine candidal vulvovaginitis and antifungal susceptibility. J Fungi. 2023;9(12):1145. https://doi.org/10.3390/jof9121145
67. Sidstedt M, Rådström P, Hedman J. PCR inhibition in qPCR, dPCR and MPS-mechanisms and solutions. Anal Bioanal Chem. 2020 Apr;412(9):2009-2023.
68. Chowdhary A, Sharma C, Meis JF. Candida auris: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog. 2017 May 18;13(5):e1006290.
69. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, Colombo AL, Calvo B, Cuomo CA, Desjardins CA, Berkow EL, Castanheira M, Magobo RE, Jabeen K, Asghar RJ, Meis JF, Jackson B, Chiller T, Litvintseva AP. Simultaneous Emergence of Multidrug-Resistant Candida auris on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses. Clin Infect Dis. 2017 Jan 15;64(2):134-140.
70. Rybak JM, Cuomo CA, Rogers PD. The molecular and genetic basis of antifungal resistance in the emerging fungal pathogen Candida auris. Curr Opin Microbiol. 2022 Dec;70:102208.
71. Barantsevich N, Barantsevich E. Diagnosis and Treatment of Invasive Candidiasis. Antibiotics (Basel). 2022 May 26;11(6):718.
72. Zuza-Alves DL, Silva-Rocha WP, Chaves GM. An Update on Candida tropicalis Based on Basic and Clinical Approaches. Front Microbiol. 2017 Oct 13;8:1927.
73. Berkow EL, Lockhart SR. Fluconazole resistance in Candida species: a current perspective. Infect Drug Resist. 2017 Jul 31;10:237-245.
74. Branco J, Miranda IM, Rodrigues AG. Candida parapsilosis Virulence and Antifungal Resistance Mechanisms: A Comprehensive Review of Key Determinants. Journal of Fungi. 2023;9(1):80. https://doi.org/10.3390/jof9010080
75. Rosa DD, Pasqualotto AC, Denning DW. Chronic mucocutaneous candidiasis and oesophageal cancer. Med Mycol. 2008 Feb;46(1):85-91. https://doi.org/10.1080/13693780701616023
76. Frías-De-León MG, Hernández-Castro R, Conde-Cuevas E, García-Coronel IH, Vázquez-Aceituno VA, Soriano-Ursúa MA, Farfán-García ED, Ocharán-Hernández E, Rodríguez-Cerdeira C, Arenas R, Robledo-Cayetano M, Ramírez-Lozada T, Meza-Meneses P, Pinto-Almazán R, Martínez-Herrera E. Candida glabrata Antifungal Resistance and Virulence Factors, a Perfect Pathogenic Combination. Pharmaceutics. 2021 Sep 22;13(10):1529.
77. Jamiu AT, Albertyn J, Sebolai OM, Pohl CH. Update on Candida krusei, a potential multidrug-resistant pathogen. Medical Mycology. 2021 Jan;59(1):14–30.
78. Bhattacharya S, Sae-Tia S, Fries BC. Candidiasis and mechanisms of antifungal resistance. Antibiotics. 2020;9(6):312. https://doi.org/10.3390/antibiotics9060312
79. Chaabane F, Graf A, Jequier L, Coste AT. Review on antifungal resistance mechanisms in the emerging pathogen Candida auris. Front Microbiol. 2019 Nov 29;10:2788.
80. Martín R, Miquel S, Langella P, Bermúdez-Humarán LG. The role of metagenomics in understanding the human microbiome in health and disease. Virulence. 2014 Apr 1;5(3):413-23.
81. O'Meara TR, Robbins N, Cowen LE. The Hsp90 Chaperone Network Modulates Candida Virulence Traits. Trends Microbiol. 2017 Oct;25(10):809-819.
82. Wijayawardene NN, Boonyuen N, Ranaweera CB, de Zoysa HKS, Padmathilake RE, Nifla F, Dai D-Q, Liu Y, Suwannarach N, Kumla J, et al. OMICS and Other Advanced Technologies in Mycological Applications. Journal of Fungi. 2023;9(6):688. https://doi.org/10.3390/jof9060688

83. Finn RD, Attwood TK, Babbitt PC, Bateman A, Bork P, Bridge AJ, Chang HY, Dosztányi Z, El-Gebali S, Fraser M, Gough J, Haft D, Holliday GL, Huang H, Huang X, Letunic I, Lopez R, Lu S, Marchler-Bauer A, Mi H, Mistry J, Natale DA, Necci M, Nuka G, Orengo CA, Park Y, Pesseat S, Piovesan D, Potter SC, Rawlings ND, Redaschi N, Richardson L, Rivoire C, Sangrador-Vegas A, Sigrist C, Sillitoe I, Smithers B, Squizzato S, Sutton G, Thanki N, Thomas PD, Tosatto SC, Wu CH, Xenarios I, Yeh LS, Young SY, Mitchell AL. InterPro in 2017-beyond protein family and domain annotations. Nucleic Acids Res. 2017 Jan 4;45(D1):D190-D199.