Exploring rapid molecular methods for diagnosing *Candida* species infecting humans: A narrative review

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**ABSTRACT**

Background: *Candida* species are perilous fungal pathogens that can cause various human infections. Accurate and timely identification of these fungi is crucial for appropriate treatment selection and effective disease management. Traditional methods for *Candida* species identification and characterization, such as phenotypic assays and culture-based techniques, have limitations in accuracy, time-consuming processes, and limited species differentiation. In recent years, molecular methods have emerged as powerful tools for rapid and accurate identification and characterization of *Candida* species. The molecular methods developed and employed for identifying and characterizing *Candida* species. It begins by highlighting the challenges associated with conventional methods and the need for more efficient and reliable techniques. The abstract then explores various molecular approaches, including polymerase chain reaction (PCR) assays, DNA sequencing, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). These methods leverage the genetic and proteomic characteristics of *Candida* species to provide accurate and specific identification and strain differentiation. Furthermore, this article discusses the application of molecular methods in clinical settings, epidemiological studies, and antifungal resistance monitoring. Molecular techniques enable rapid detection of *Candida* species directly from clinical samples, facilitating early diagnosis and prompt initiation of appropriate antifungal therapy. They also play a crucial role in epidemiological investigations, allowing for the identification of clonal outbreaks and the tracking of transmission patterns. Additionally, molecular methods aid in detecting genetic markers associated with antifungal resistance, enabling tailored therapeutic approaches.

**Introduction**

*Candida* species are essential opportunistic fungal pathogens that cause many human infections. Accurate identification and characterization of *Candida* species are crucial for effective patient management, appropriate antifungal therapy selection, and understanding the epidemiology of candidiasis. Traditional *Candida* species identification and characterization methods, such as phenotypic assays and culture-based techniques, have accuracy, turnaround time, and species differentiation limitations. Therefore, the development and application of molecular
methods have revolutionized the field by providing rapid, accurate, and comprehensive approaches for Candida species identification and characterization. This review explores the molecular methods developed and utilized for Candida species identification and characterization. These methods leverage the genetic and proteomic characteristics of Candida species to improve accuracy and speed in species identification and strain differentiation [1-3].

**Candida species**

Candida species belong to the kingdom Fungi, phylum Ascomycota, class Saccharomyces, family Saccharomycetaceae, and genus Candida. These yeasts are unicellular microorganisms that exhibit pleomorphic shapes, are oval or spherical, and have an incomplete sexual cycle. Candida species are commonly found as commensal inhabitants of the human body, residing in healthy individuals' respiratory tract, gastrointestinal tract, vaginal mucosa, oral cavity, and skin (Figure 1, 4). They can also be found in plants, water, soil, and other environments. These versatile organisms can degrade proteins and carbohydrates, utilizing them as essential carbon and nitrogen sources for their growth [5].

**Figure 1.** Common fungal pathogens affecting different parts of the body

The interaction between a native Candida species and its human host can be influenced by various factors, including pathological, physiological, mechanical, and iatrogenic factors. Consequently, Candida species can cause a wide range of infections with diverse clinical manifestations, ranging from simple and benign forms to invasive infections that can affect multiple organs and ultimately result in the host's death [6]. Among the nearly 200 different Candida species, a small number of them are of clinical significance. The most prevalent ones include C. albicans, C. tropicalis, C. glabrata, C. krusei, and C. parapsilosis (which include C. orthopsilosis and C. metapsilosis). These species are responsible for over 90% of invasive Candida infections. Additionally, there are emerging Candida species, such as C. guilliermondii, C. dubliniensis, C. lusitaniae, C. kefir, C. rugosa, C. famata, C. utilise, C. lipolytica, C. norvegensis, and C. inconspicua, which have clinical relevance and have been identified as causative agents of both superficial and systemic infections [7].

**Prominent Candida species involved in Infections**

Several Candida species have been identified as significant pathogens in various clinical infections. Understanding these species' prevalence and clinical significance is essential for accurately diagnosing and effectively managing Candida infections. Among the prominent infection species, C. albicans remains the most common and clinically relevant species [8]. It accounts for many superficial and invasive Candida infections, including candidemia, oral thrush, and vaginal candidiasis. C. tropicalis is another important species associated with invasive candidiasis, particularly in immunocompromised patients [9]. It has been found to exhibit intrinsic resistance to certain antifungal drugs, highlighting the need for
accurate identification and appropriate treatment selection. *C. glabrata* has emerged as a significant pathogen, especially in nosocomial infections and among immunocompromised individuals [10]. It is known for its ability to develop resistance to commonly used antifungal agents, posing challenges in treatment.

*C. krusei*, although less prevalent than other species, is noteworthy due to its inherent resistance to fluconazole, a commonly prescribed antifungal drug [5-10]. This species is often associated with infections in patients with prior exposure to antifungal agents. *C. parapsilosis*, consisting of three distinct subgroups (*C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*), is increasingly recognised as a cause of healthcare-associated infections, particularly in neonates and patients with indwelling medical devices [5-10].

**Clinical relevance and role of Candida species in various types of infections**

*Candida* species play a significant role in a wide range of infections, from superficial to invasive and systemic (Figure 2). Understanding their clinical relevance in different 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.

**Invasive candidiasis:** *Candida* species, including *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*, can cause invasive candidiasis, invading deeper tissues and organs. This includes candidemia (bloodstream infection), disseminated candidiasis, and deep-seated organ infections. Invasive candidiasis typically occurs in immunocompromised individuals, such as those with compromised immune systems, critically ill patients, or those undergoing invasive medical procedures [11].

**Candida-associated urinary tract infections (UTIs):** *Candida* species can also colonise and infect the urinary tract, leading to UTIs. *C. albicans* is the most common species associated with urinary tract infections, although other species like *C. glabrata* and *C. tropicalis* can also be involved. Candida UTIs are more prevalent in patients with indwelling urinary catheters or underlying urinary tract abnormalities [12].

**Gastrointestinal candidiasis:** *Candida* species, especially *C. 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 [13].

**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 body areas, such as skin folds or between the toes. They are...
frequently associated with excessive moisture, poor hygiene, or compromised skin integrity [14].

It is important to note that the clinical relevance of *Candida* species can vary depending on geographical location, patient population, and underlying risk factors. Proper identification of the *Candida* involved in specific infections is essential for targeted and adequate antifungal therapy.

**Importance of accurate *Candida* species identification**

Accurate identification of *Candida* species is essential for several reasons, including effective patient management, appropriate antifungal therapy selection, and understanding the epidemiology of candidiasis. Accurately identifying *Candida* species allows clinicians to make informed decisions regarding treatment strategies, leading to improved patient outcomes.

**Impact on patient management:** Accurate species identification helps determine the appropriate antifungal therapy for candidiasis. Different *Candida* species may exhibit varying susceptibilities to antifungal agents. For example, *C. glabrata* and *C. krusei* are known to be less susceptible to azole antifungals, while *C. albicans* are generally more susceptible. Therefore, accurate identification allows tailored treatment approaches based on the specific *Candida* species involved [15].

**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 *C. glabrata* or *C. krusei*, while azoles are commonly used for *C. albicans* infections. Accurate identification of the causative species enables the selection of the most appropriate antifungal therapy, optimising treatment outcomes [16, 17].

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 and 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. These limitations highlight the need for more advanced molecular methods in *Candida* species identification [20-22].

**Phenotypic assays**

Phenotypic assays rely on observing various characteristics, such as colony morphology, biochemical reactions, and growth at different temperatures or on specific media (Figure 3). However, these methods may need more specificity and accuracy, leading to misidentification or difficulty distinguishing closely related *Candida* species [23]. Furthermore, phenotypic assays may require extended incubation periods, resulting in delays in obtaining conclusive identification. Culture-based techniques involve the growth of *Candida* isolates on specific agar media followed by a morphological examination. While these methods have been traditionally used, they have limitations, including requiring skilled laboratory personnel, time-consuming procedures, and the potential for contamination or overgrowth by other microorganisms [24].

**Figure 3. Candida isolation on culture method**
Challenges in species differentiation: Some *Candida* species exhibit similar morphological and biochemical characteristics, making their differentiation challenging using conventional methods alone. For example, distinguishing *C. dubliniensis* from *C. albicans* or accurately identifying emerging species with clinical significance, such as *C. auris*, can be challenging [25, 26].

Accuracy in detecting mixed infections: Traditional methods may need help to detect mixed *Candida* infections accurately, where more than one species coexists. These infections can be particularly problematic as different species may respond differently to treatment and have varying degrees of pathogenicity [27].

**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* to enable precise identification and characterization. The following provides an overview of some commonly used molecular methods for *Candida* identification:

- **Polymerase Chain Reaction (PCR) assays**: PCR assays are widely employed for detecting and identifying *Candida* species. These methods amplify specific regions of the 9-genome, such as the internal transcribed spacer (ITS) region, to generate DNA fragments that can be analyzed. PCR-based assays offer high sensitivity, specificity, and speed, allowing for the rapid identification of *Candida* from various clinical specimens [28].

- **DNA sequencing techniques**: DNA sequencing methods, including Sanger 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 [29].

- **Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)**: MALDI-TOF MS has emerged as a powerful tool for rapidly identifying *Candida* species. It relies on detecting 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 [30].

  **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 beneficial for epidemiological studies and tracking the spread of *Candida* infections [31].

**Advantages of molecular methods over traditional approaches**

Molecular methods for *Candida* species identification offer several advantages over traditional approaches, providing more accurate and efficient results. The following are some critical advantages of molecular methods:

- **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 with similar phenotypic characteristics, reducing the chances of misidentification [32]. 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 than traditional techniques. Techniques such as PCR assays and MALDI-TOF MS can identify species within hours, allowing for timely decision-making regarding patient management and appropriate antifungal therapy selection [31]. 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 [28-31]. This enhanced sensitivity enables the detection of *Candida* species that conventional culture-based methods may have otherwise missed.

Detection of mixed infections: Molecular methods are valuable in detecting mixed *Candida* infections, where multiple species coexist. By amplifying and analyzing specific genetic targets, these methods can identify and differentiate multiple *Candida* in a single clinical sample, aiding in tailored treatment approaches [27]. This capability is particularly beneficial in complex clinical scenarios and immunocompromised patients.

Table 1. Summary of pros and cons in rapid detection methods for *Candida* species

<table>
<thead>
<tr>
<th>Diagnostic approaches</th>
<th>Advantages</th>
<th>Limitations</th>
<th>References</th>
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<tr>
<td>Pulsed-field gel electrophoresis (PFGE)</td>
<td>precise <em>candida</em> strain discrimination.</td>
<td>labor-intensive and time-consuming analysis process.</td>
<td>[36-38]</td>
</tr>
<tr>
<td>Restriction enzyme analysis (REA)</td>
<td>specific genetic marker identification.</td>
<td>difficulty in identifying specific genes.</td>
<td>[39]</td>
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<tr>
<td>Random amplified polymorphic DNA (RAPD)</td>
<td>genetic diversity profiling in <em>candida</em>.</td>
<td>limited discrimination among genetically similar strains.</td>
<td>[40, 41]</td>
</tr>
<tr>
<td>Amplified fragment length polymorphism (AFLP)</td>
<td>high-resolution genomic fingerprinting in <em>candida</em>.</td>
<td>challenging data interpretation and standardization issues.</td>
<td>[42, 43]</td>
</tr>
<tr>
<td>Nested PCR</td>
<td>increased sensitivity in <em>candida</em> detection.</td>
<td>potential amplification bias with nested PCR.</td>
<td>[44]</td>
</tr>
<tr>
<td>Real-time PCR (RT-PCR)</td>
<td>rapid and accurate <em>candida</em> detection.</td>
<td>risk of contamination in sensitive assays.</td>
<td>[45, 46]</td>
</tr>
<tr>
<td>Nucleic acid sequence-based amplification (NASBA)</td>
<td>sensitive and specific <em>candida</em> detection.</td>
<td>sensitivity affected by sample quality.</td>
<td>[47]</td>
</tr>
<tr>
<td>Peptide nucleic acid-fluorescent in situ hybridization (PNA-FISH)</td>
<td>rapid and targeted <em>candida</em> identification.</td>
<td>limited discrimination among closely related species.</td>
<td>[44, 48]</td>
</tr>
<tr>
<td>Microsatellite length polymorphism (MLP) typing</td>
<td>high-resolution <em>candida</em> strain typing.</td>
<td>requires customized primer design for analysis.</td>
<td>[49]</td>
</tr>
<tr>
<td>Multi-locus sequence typing (MLST)</td>
<td>precise genetic characterization in <em>candida</em> identification.</td>
<td>time-consuming and labor-intensive technique.</td>
<td>[44]</td>
</tr>
<tr>
<td>DNA-microarrays</td>
<td>simultaneous detection of multiple <em>candida</em> species.</td>
<td>dependency on specific genomic databases.</td>
<td>[48, 49]</td>
</tr>
<tr>
<td>Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)</td>
<td>rapid and accurate <em>candida</em> species identification.</td>
<td>limited database for rare <em>candida</em> species.</td>
<td>[49]</td>
</tr>
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**Application of molecular methods in epidemiological studies**

Molecular methods have proven valuable in epidemiological studies focused on understanding *Candida* transmission, spread, and genetic diversity. The application of these methods provides essential 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 identifying clonal clusters and genetic relatedness and tracking specific strains within and between healthcare facilities [30, 31].

Outbreak investigation: Molecular methods play a crucial role in identifying and investigating outbreaks of *Candida* infections. By comparing the genetic profiles of *Candida* isolates from different patients, healthcare workers, or environmental sources, molecular
methods can determine if the cases are linked and establish the outbreak's source [28, 29]. This information helps implement appropriate infection control measures and prevent further spread.

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 [33, 34]. This knowledge is essential for monitoring trends in resistance and optimizing antifungal treatment strategies.

Population structure and evolutionary analysis: Molecular methods allow the study of *Candida* population structure and evolutionary relationships. By analyzing 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 [33, 34]. This information is crucial for understanding the epidemiology of *candida* infections and designing targeted control measures.

**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 advances, several exciting future directions and potential advancements in molecular methods for *Candida* identification exists.

Next-generation sequencing (NGS): NGS technologies, such as whole-genome sequencing (WGS), hold great promise for *Candida* identification and characterization. WGS allows for comprehensive genomic analysis, including identifying genetic variations, virulence factors, and antifungal resistance genes, providing a deeper understanding of *Candida* species’ pathogenicity and epidemiology [35].

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 [36].

Point-of-care testing (POCT): The 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 [50].

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 antifungal resistance mechanisms [51].

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 [52-54].

**Conclusion**

These advancements can potentially revolutionize the *Candida* species identification and characterization field, leading to improved diagnostics, personalized treatment strategies, and better management of *Candida* infections.

**Conflicts of interests**

The authors declare there are no conflicts of interest.

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