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Review article

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

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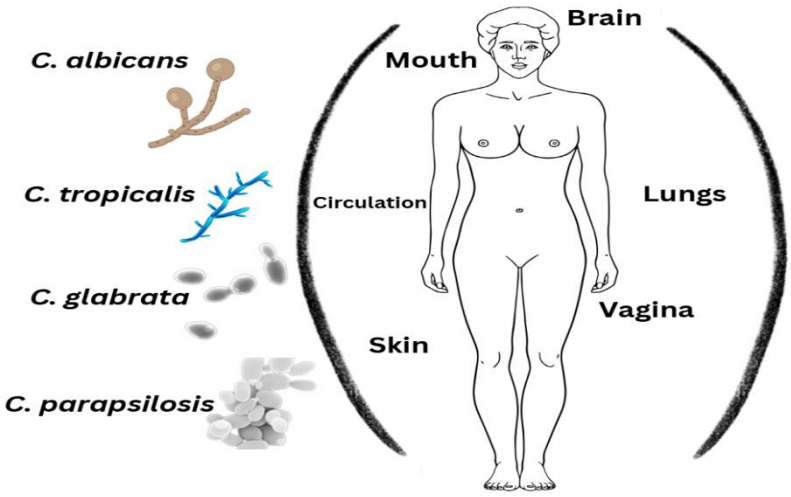
methods have revolutionized the field by providing rapid, accurate, and comprehensive approaches for *Candida* species identification and characterization. This review explores the molecular methods developed and utilized for *Candida* species identification and characterization. These methods leverage 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.

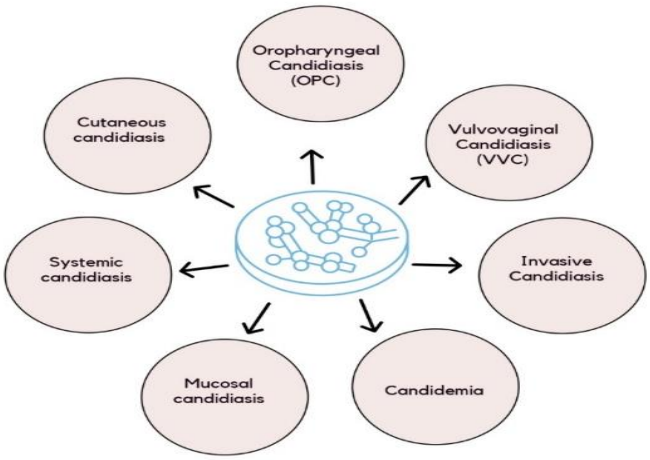
C. krusei, although less prevalent than other species, is noteworthy due to its inherent resistance to fluconazole, a commonly prescribed antifungal drug [5-10]. This species is often associated with infections in patients with prior exposure to antifungal agents. *C. parapsilosis*, consisting of three 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].

Figure 2. Different types of Candida infections



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 immuno-compromised patients.

Potential for simultaneous detection of resistance markers: Molecular methods can also be employed to detect antifungal resistance markers in *Candida*. This enables the identification of drug-resistant strains and helps guide appropriate antifungal therapy selection [33, 34]. Combining species identification with resistance detection in a single assay provides a comprehensive understanding of the clinical isolate's characteristics. **Table (1)** summarizes all the rapid detection methods for *Candida* species and their pros and cons.

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

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

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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References

1-**Spampinato C, Leonardi D.** *Candida* infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. Biomed Res Int 2013; 2013:204237.

2- **Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS.** *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol 2013;62(Pt 1):10-24.

3- **Papon N, Courdavault V, Clastre M, Bennett RJ.** Emerging and emerged pathogenic *Candida* species: beyond the Candida albicans paradigm. PLoS Pathog 2013;9(9): e1003550. doi: 10.1371/ journal. ppat.1003550.

4- **Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC.** Hidden killers: human fungal infections. Sci Transl Med 2012;4(165):165rv13.

5- **Turner SA, Butler G.** The *Candida* pathogenic species complex. Cold Spring Harb Perspect Med 2014;4(9): a019778.

6- **Mohandas V, Ballal M.** Distribution of *Candida* species in different clinical samples and their virulence: biofilm formation, proteinase and phospholipase production: a study on hospitalized patients in southern India. J Glob Infect Dis 2011;3(1):4-8.

7- **R AN, Rafiq NB.** Candidiasis. 2023 May 29. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 32809459.

8- **Turner SA, Butler G.** The *Candida* pathogenic species complex. Cold Spring Harb Perspect Med 2014;4(9): a019778.

9- **de Oliveira Santos GC, Vasconcelos CC, Lopes AJO, de Sousa Cartágenes MDS, Filho AKDB, do Nascimento FRF, et al.** *Candida* Infections and Therapeutic Strategies: Mechanisms of Action for Traditional and Alternative Agents. Front Microbiol 2018; 9:1351.

10- **Kabir MA, Ahmad Z.** *Candida* infections and their prevention. ISRN Prev Med 2012; 2013:763628.

11- **Hay RJ.** The management of superficial candidiasis. J Am Acad Dermatol 1999;40(6 Pt 2): S35-42.

12- **Behzadi P, Behzadi E, Ranjbar R**. Urinary tract infections and *Candida albicans*. Cent European J Urol 2015;68(1):96-101.

13- **Alonso-Monge R, Gresnigt MS, Román E, Hube B, Pla J.** *Candida albicans* colonization of the gastrointestinal tract: A double-edged sword. PLoS Pathog 2021 22;17(7): e1009710.

14- **Leung AKC, Lam JM, Leong KF, Hon KL, Barankin B, Leung AAM, et al**. Onychomycosis: An Updated Review. Recent Pat Inflamm Allergy Drug Discov 2020;14(1):32-45.

15- **Lockhart SR, Jackson BR, Vallabhaneni S, Ostrosky-Zeichner L, Pappas PG, Chiller T.** Thinking beyond the Common *Candida* Species: Need for Species-Level Identification of *Candida* Due to the Emergence of Multidrug-Resistant *Candida* auris. J Clin Microbiol 2017;55(12):3324-3327.

16- **Mazu TK, Bricker BA, Flores-Rozas H, Ablordeppey SY.** The Mechanistic Targets of Antifungal Agents: An Overview. Mini Rev Med Chem 2016;16(7):555-78.

17- **Mota Fernandes C, Dasilva D, Haranahalli K, McCarthy JB, Mallamo J, Ojima I, et al.** The Future of Antifungal Drug Therapy: Novel Compounds and Targets. Antimicrob Agents Chemother 2021 20;65(2): e01719-20.

18- **Bhattacharjee P.** Epidemiology and antifungal susceptibility of *Candida* species

in a tertiary care hospital, Kolkata, India. Curr Med Mycol 2016;2(2):20-27.

19-**Zhang W, Song X, Wu H, Zheng R.** Epidemiology, risk factors and outcomes of *Candida albicans* vs. non-albicans candidaemia in adult patients in Northeast China. Epidemiol Infect 2019 25;147: e277.

20-**Yeo SF, Wong B**. Current status of nonculture methods for diagnosis of invasive fungal infections. Clin Microbiol Rev 2002;15(3):465-84.

21-**Alam MZ, Alam Q, Jiman-Fatani A, Kamal MA, Abuzenadah AM, Chaudhary AG, et al.** *Candida* identification: a journey from conventional to molecular methods in medical mycology. World J Microbiol Biotechnol 2014;30(5):1437-51.

22-**Souza MN, Ortiz SO, Mello MM, Oliveira Fde M, Severo LC, Goebel CS**. Comparison between four usual methods of identification of *Candida* species. Rev Inst Med Trop Sao Paulo 2015;57(4):281-7.

23-**Marinho SA, Teixeira AB, Santos OS, Cazanova RF, Ferreira CA, et al.** Identification of *Candida* spp. by phenotypic tests and PCR. Braz J Microbiol 2010;41(2):286-94.

24-**Safavieh M, Coarsey C, Esiobu N, Memic A, Vyas JM, Shafiee H, et al.** Advances in *Candida* detection platforms for clinical and point-of-care applications. Crit Rev Biotechnol 2017;37(4):441-458.

25-**Riera FO, Caeiro JP, Angiolini SC, Vigezzi C, Rodriguez E, Icely PA, et al.** Invasive Candidiasis: Update and current challenges in the management of this mycosis in South America. Antibiotics (Basel) 2022 30;11(7):877.

26-**Fang W, Wu J, Cheng M, Zhu X, Du M, Chen C, et al.** Diagnosis of invasive fungal infections: challenges and recent developments. J Biomed Sci 2023 19;30(1):42.

27-**Farooq H, Monowar T, V Chinni S, Swe Latt S, Hasliza Zainol N, Shankar Sabesan G**. Epidemiology, and molecular identification of mixed yeast isolates in Malaysia: A way forward. Curr Med Mycol 2022;8(3):35-38.

28-**Eghtedar N E, Ghasemi N, Almani P, Mohammadi MA, Salari S.** Molecular identification of *Candida* isolates by Real-time PCR-high-resolution melting analysis and investigation of the genetic diversity of *Candida* species. J Clin Lab Anal 2020;34(10): e23444.

29-**Barantsevich N, Barantsevich E.** Diagnosis and Treatment of Invasive Candidiasis. Antibiotics (Basel) 2022;11(6):718.

30-**De Carolis E, Vella A, Vaccaro L, Torelli R, Spanu T, Fiori B, et al.** Application of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. J Infect Dev Ctries 2014;8(9):1081-8.

31-**Delavy M, Dos Santos AR, Heiman CM, Coste AT.** Investigating Antifungal Susceptibility in *Candida* Species With MALDI-TOF MS-Based Assays. Front Cell Infect Microbiol 2019; 9:19.

32-**Kidd SE, Chen SC, Meyer W, Halliday CL. A** New Age in Molecular Diagnostics for Invasive Fungal Disease: Are We Ready? Front Microbiol 2020; 10:2903.25.

33-**Elie CM, Lott TJ, Reiss E, Morrison CJ.** Rapid identification of *Candida* species with species-specific DNA probes. J Clin Microbiol 1998;36(11):3260-5.

34- **Garcia-Effron G.** Molecular Markers of Antifungal Resistance: Potential Uses in Routine Practice and Future Perspectives. J Fungi (Basel) 2021;7(3):197.

35- **Jiang S, Chen Y, Han S, Lv L, Li L.** Next-Generation Sequencing Applications for the Study of Fungal Pathogens. Microorganisms 2022;10(10):1882

36- **Magee BB, Magee PT.** Electrophoretic karyotypes and chromosome numbers in *Candida* species. J Gen Microbiol 1987; 133:425–430.

37- **Hahm BK, Maldonado Y, Schreiber E, Bhunia AK, Nakatsu CH.** Subtyping of foodborne and environmental isolates of *Escherichia coli* by multiplex-PCR, rep-PCR, PFGE, ribotyping and AFLP. J Microbiol Methods 2003; 53:387–399.

38- **Olive DM, Bean P.** Principles and applications of methods for DNA-based typing of microbial organisms. J Clin Microbiol 1999; 37:1661–1669.

39- **Lehmann PF, Lin D, Lasker BA.** Genotypic identification and characterization of species and strains within the genus *Candida* by using random amplified polymorphic DNA. J Clin Microbiol 1992; 30:3249–3254.

40- **Tabit FT.** Advantages and limitations of potential methods for the analysis of bacteria in milk: a review. J Food Sci Technol 2016 53:42–49.

41- **Ball LM, Bes MA, Theelen B, Boekhout T, Egeler RM, Kuijper EJ.** Significance of amplified fragment length polymorphism in identification and epidemiological examination of *Candida* species colonization in children undergoing allogeneic stem cell transplantation. J Clin Microbiol 2004; 42:1673–1679.

42-**Hsu MC, Chen KW, Lo HJ, Chen YC, Liao MH, Lin YH, et al.** Species identification of medically important fungi by use of real-time light cycler PCR. J Med Microbiol 2003; 52:1071–1076.

43-**Magalhães 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; 12:9204.

44-**Widjojoatmodjo MN, Borst A, Schukkink RAF, Box ATA, Tacken NMM, Gemen BV, et al.** Nucleic acid sequence-based amplification (NASBA) detection of medically important *Candida* species. J Microbiol Methods 1999; 38:81–90.

45-**Kempf VAJ, Trebesius K, Autenrieth IB.** Fluorescent in situ hybridization allows rapid identification of microorganisms in blood cultures. J Clin Microbiol 2000; 38:830–838.

46-**Sampaio P, Gusmao L, Correia A, Alves C, Rodrigues AG, PinaVaz C, et al.** New microsatellite multiplex PCR for *Candida* albicans strain typing reveals microevolutionary changes. J Clin Microbiol 2005; 43:3869–3876.

47-**Garcia-Hermoso D, Cabaret O, Lecellier G, Desnos-Ollivier M, Hoinard D, Raoux D, et al.** Comparison of microsatellite length polymorphism and multilocus sequence typing for DNA-based typing of *Candida* albicans. J Clin Microbiol 2007; 45:3958–3963.

48-**Kurella M, Hsiao LL, Yoshida T, Randall JD, Chow G, Sarang SS, et al**. DNA microarray analysis of complex biologic processes. J Am Soc Nephrol 2001; 12:1072–1078.

49-**Kittichotirat W, Bumgarner RE, Asikainen S, Chen C.** Identification of the

pangenome and its components in 14 distinct *Aggregatibacter actinomycetemcomitans* strains by comparative genomic analysis. PLoS ONE 2011; 6: e22420.

50-**O'Meara TR.** Metagenomic Sequencing for Direct Identification of *Candida auris* Colonization. mSphere 2021;6(4): e0063821.

51-**Osaigbovo II, Bongomin F.** Point of care tests for invasive fungal infections: a blueprint for increasing availability in Africa. Ther Adv Infect Dis 2021; 8:20499361211034266.

52-**Min K, Jannace TF, Si H, Veeramah KR, Haley JD, Konopka JB.** Integrative multi-omics profiling reveals cAMP-independent mechanisms regulating hyphal morphogenesis in *Candida albicans*. PLoS Pathog 2021;17(8): e1009861.

53-**Zhu GD, Xie LM, Su JW, Cao XJ, Yin X, Li YP, et al.** Identification of differentially expressed genes and signaling pathways with *Candida* infection by bioinformatics analysis. Eur J Med Res 2022;27(1):43.

54-**Naik S, Mohammed A.** Coexpression network analysis of human *candida* infection reveals key modules and hub genes responsible for host-pathogen interactions. Front Genet 2022; 13:917636.

Dubey D, Rath S, Subhadarshini S S, Purohit G K, Tripathy D, Panigrahi, Palai S, SSamanta D. Exploring rapid molecular methods for diagnosing *Candida* Species infecting humans: A narrative review. Microbes Infect Dis 2024; 5(1): 336-346.