Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 792-796
DoI-10.004687/1000-9035.2025.107

Journal of Molecular Science

Journal of Molecular Science
www.jmolecularsci.com ISSN:1000-9035

Standardization of simple low-cost Conventional PCR for the identification of *Candida parapsilosis* species complex

R Hari Presanambika[1], Vijayakishore Thanneru[2], Kanagasabapathi Karthika[3], Thayanidhi Premamalini[*], Anupma Jyoti Kindo[4]
[1,2,3,4]Department of Microbiology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamilnadu, 600106, India.
[5]Professor, Department of Microbiology, Sri Ramachandra Medical College & Research Institute, SRIHER, Porur, Chennai, Tamilnadu, 600106, India.

Article Information
Received: 12-09-2025
Revised: 07-10-2025
Accepted: 29-10-2025
Published: 19-11-2025

Keywords
Candida parapsilosis complex, Complex specific primers, Early diagnosis, Polymerase Chain Reaction.

ABSTRACT

Background and objectives: *Candida parapsilosis* complex have been reported as the second most common species isolated from patients with invasive candidiasis. Although conventional phenotypic techniques are available, in practice, many other *Candida* species are also known to produce similar phenotypic presentations. Hence, confirmation of the species cannot be relied upon by these methods. Therefore, there is a need for molecular methods which will help in the accurate identification of this complex.

Materials and methods: All *Candida* isolates received in the laboratory during the study period were subjected to phenotypic identification. It was confirmed by Genotypic identification techniques like Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) using pan fungal primers and restriction enzyme. Species specific primers were designed and the PCR was standardized for early identification of *C. parapsilosis* isolates.

Results: About 14.9% (n=58) of the isolates were identified as *C. parapsilosis* complex. The designed primers were tested against all the clinical isolates as well as the control strains and it produced band only for *C. parapsilosis* complex at 362 bp whereas it did not produce band for other *Candida* species (Negative control), thus, validating our result. Further, the representative isolates have been confirmed by gene sequencing which showed 100% concordance with our results for *C. parapsilosis* complex identification.

Conclusion: This simple, low-cost conventional PCR assay performed directly from clinical isolate aids in early diagnosis in less than 3 hours, allowing timely treatment and management of infections. It also helps to ascertain the prevalence database for epidemiological studies and outbreak investigations.

INTRODUCTION:

The importance of non-albicans *Candida* has been increasing in the past decade mainly due to the emergence of new resistance species of *Candida* and also due to the improved diagnostic facilities available worldwide. Of the non-albicans *Candida* (NAC) species *C. tropicalis*, *C. parapsilosis* complex and *C. glabrata* are some of the commonly isolated species (1).

C. parapsilosis complex is one of the most common *Candida* species causing invasive diseases worldwide. They are commensals of human skin but are known to cause invasive infections like endocarditis, meningitis, peritonitis etc (2). Previously classified into group I, II, III, now they are given specific species names *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* respectively. They are collectively called *Candida parapsilosis* complex (3). They are three distinct but closely related species. Over the past 20 years, human infections brought on by the *Candida parapsilosis* species complex have significantly increased in importance and frequency (4). Their capacity to form biofilms and extracellular slime helps in their attachment to catheters and prosthetic devices. They also secrete enzymes like phospholipases, protease, lipases and other hydrolytic enzymes. All these factors contribute to the high virulence and pathogenicity of the *C. parapsilosis* complex among NAC species (1). They are most commonly implicated in infections associated with indwelling catheters, hyperalimentation solutions, medical devices or fluids, prosthetic devices, steroids, H2 blockers and immunocompromised conditions like neutropenia, AIDS. They are found on the hands of health care workers which serves as a source of nosocomial outbreaks (Govrins & Lass- Flörl, 2024).

In recent studies, *C. parapsilosis* complex were found to show increased resistance to azoles compared to echinocandins. Cross resistance between antifungals were also noted in *C. parapsilosis* complex in the outbreak isolates (6,7,8). Hence molecular identification of *C. parapsilosis* complex will help clinicians in the choice of drug by correct identification and also help to ascertain the prevalence database for epidemiological purposes. Although conventional phenotypic identification techniques are available for the identification of *C. parapsilosis* complex. They are laborious, time consuming and confirmation of the species cannot be relied upon by these methods. Therefore, there is a need for molecular methods which will help in the accurate identification of the species complex.

MATERIALS AND METHODS:

This study was conducted from August 2023 to July 2024 in the Department of Microbiology in a tertiary care center after obtaining an institutional ethical clearance. All 388 *Candida* isolates obtained from various clinical samples like catheter tip, blood, urine, tissue, pus, nasal swab was used for the study. All the samples that grew yeast were initially identified by conventional phenotypic techniques and it was further confirmed by genotypic identification techniques with appropriate control strains.

Presumptive phenotypic identification of *Candida* species:

The samples received were initially subjected to gram staining. Germ tube test was performed to rule out *C. albicans* from NAC species. All the isolates were subcultured on Sabouraud's Dextrose Agar (SDA) for maintenance of the isolates and chromogenic media like Triphenyl Tetrazolium medium (TTZ) and HiCrome agar to differentiate from other *Candida* species. All the isolates producing cream to pink color colonies in HiCrome agar were presumptively identified as *C. parapsilosis* complex. Sugar assimilation and fermentation tests were also done for differentiation.

Molecular Identification Techniques:

Genotypic identification was done by extracting DNA using in-house phenol chloroform method with minor modifications. This was followed by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) using Pan fungal primers and *Msp* I restriction enzyme (9,10).

Polymerase Chain Reaction (PCR):

The PCR amplification of the internal transcribed spacer region was carried out using universal fungal primers i.e., ITS 1 (5' – TCC GTA GGT GAA CCT GCG G – 3') and ITS 4 (5' – TCC TCC GCT TAT TGA TAT GC – 3'. The PCR master mix was prepared containing 25 µl of PCR mix (Takara, Japan), 1 µl of forward and reverse primer, 5 µl of template DNA, and the volume was increased with sterile nuclease-free water to reach 50 µl. The reaction mixtures were amplified in a thermal cycler (Veriti 96 well, Applied Biosystems, USA), with the following reaction conditions: at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 sec, 56 °C for 30 sec, and 72 °C for 30 sec, with a final extension period at 72 °C for 10 min. After amplification, 10 µl of the PCR product was subjected to gel electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet illumination in a Bio-Rad Gel Documentation system (USA).

Restriction Fragment Length Polymorphism (PCR-RFLP):

RFLP was done by using *Msp* I enzyme (GeNei, Banglore), 2µl of 10X enzyme buffer, 10µl of PCR product and the volume was made upto to 20µl by adding nuclease free water. The mixture was incubated at 37ºC for 1 hour and 10 µl of the product was subjected to gel electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet illumination in a Bio-Rad Gel Documentation system (USA). Isolates producing band at ~520 bp region were sent for gene sequencing to confirm the identification of *C. parapsilosis* species complex.

In-house primer designing:
The nucleotide sequences of the ITS region of *C. parapsilosis* species complex were obtained from the NCBI database and were aligned using MEGA software (version 11). The primers were designed by using Primer3web software v4.1 (https://primer3.ut.ee/). It was verified by NCBI Nucleotide BLAST tool and further analysed by in-silico PCR (http://insilico.ehu.es/user_seqs/) software. The primers were synthesized by Sigma-Aldrich Chemical Pvt. Ltd., Bangalore.

Standardization of polymerase chain reaction for detection of *C. parapsilosis* complex:
PCR standardization was done using the sequence confirmed *C. parapsilosis* complex isolates which were taken as positive controls. ATCC strains of *C. albicans, C. krusei, C. tropicalis* and sequence confirmed *C. glabrata, C. guilliermondi*i, *C. auris* were used as negative controls. The in-house designed primers (RHPCPF- 5`- CAGAATGAAAAGTGCTTAACTGC -3`, RHPCPR- 5`- TCTTTCAAGCAAACCCAGCG -3`) were tested against all the clinical isolates and control strains. The reaction was carried out for 25µl, which contains 12.5µl of Master mix (TAKARA, Japan), 0.5µl of forward primer, 0.5µl of reverse primer and 11.5µl of nuclease free water. A single isolated colony was picked and mixed with the master mix. PCR amplification was carried out in a thermocycler. The following were the PCR reaction conditions for the identification of *C. parapsilosis* complex: initial denaturation at 95ºC for 5 minutes followed by 35 cycles of denaturation at 95ºC for 30 seconds, annealing at 58ºC for 30 seconds, extension at 72ºC for 30 seconds and final extension at 72ºC for 10 minutes. 10µl of the amplified products were analyzed by using 2% agarose gel electrophoresis stained with ethidium bromide, and visualized under ultraviolet illumination in a Bio-Rad Gel Documentation system (USA).

RESULTS:

A total of 388 *Candida* isolates showing budding yeast cells with and without pseudohyphae were identified by gram staining. Formation of germ tube was observed in 114 *Candida* isolates. On HiCrome agar, *C. albicans* produced green color colonies whereas *C. tropicalis* produced metallic blue colonies and remaining all other *Candida* isolates produced cream to pink, pink to purple colonies (Fig. 1). Only *C. albicans* and *C. tropicalis* were able to differentiate phenotypically by growth on HiCrome agar whereas all other isolates produced similar color pattern as *C. parapsilosis* species complex. Further, genotypic technique by PCR-RFLP identified all the clinical *Candida* isolates as follows *C. albicans*-114, *C. tropicalis*-138, *Candida parapsilosis* complex-58, *C. glabrata*-26, *C. krusei*-14 and other NAC-38 (Fig. 2 and 3).

All 388 clinical *Candida* isolates were then subjected to PCR for in-house designed *C. parapsilosis* complex specific primers. All *Candida parapsilosis* complex (n=58) isolates produced band at 362bp while no band was observed for other *Candida* species. Bright sharp bands were observed at 362 bp region for the positive controls whereas no bands were observed for the negative controls (Fig. 4). Thus, the controls were satisfactory. This conventional PCR method shows 100% concordance with the gene sequencing results which correctly identifies all *C. parapsilosis* species complex. Thus, validating our assay.

Nucleotide sequence accession numbers
The sequences obtained in this study have been provided with the following GenBank accession numbers – PQ721029, PQ721032, PQ721033, PQ722542- PQ722546.

DISCUSSION:

Recent studies have reported higher prevalence and distribution of *Candida parapsilosis* complex species from all clinical samples (11,12). In our study, the prevalence of *Candida parapsilosis* complex accounts for about 14.9%, whereas in other countries the prevalence is higher with 29.5% (13) and 36.6% (14). This shows that there is a considerable variation in the distribution of *Candida parapsilosis* complex at different geographic locations.

Phenotypic methods like growth on chromogenic media, sugar assimilation and fermentation tests for identification of *Candida* are inexpensive and simple but are subjective, requires training and are dependent on the growth medium and temperature. Moreover, they have low discriminating capacity for few *Candida* species. Hence, conventional phenotypic methods cannot be satisfactorily relied upon (15,16,17). Biochemical tests by Vitek YBC identifies 87.3% of the *Candida* species correctly after 24-48 hrs of incubation still has disadvantages that *C. sake* was misidentified as *C. parapsilosis* which may result in inappropriate management (18). In our study, 136 of 388 (35.1%) isolates were shown to have phenotypic characteristics of *C. parapsilosis* complex but only 58 out of 136 were *C. parapsilosis* complex by genotypic techniques. 57.4% (78 out of 136) isolates were phenotypically misidentified as *C. parapsilosis* species complex. Similarly, in a study conducted by Coignard C *et al* 35% of the misidentifications were *C. parapsilosis* with 14% of *C. parapsilosis* misidentified as *C. albicans* by CHROM agar and API 20C AUX system. 53% of the misidentified profiles were interpreted as "acceptable" to "low discrimination" by API 20C AUX system (19). Cordeiro *et al* in their study showed that *C. famata* and *C. gulliermondi* can phenotypically mimic *C. parapsilosis*. 6 out of 34 isolates showing cellular arrangement of *C. parapsilosis* were found to be *C. gulliermondi* by DNA sequencing (20). However, in our study, *C. gulliermondi* control strain (negative control) produced no band with our in-house designed complex specific primers which shows high specificity of the standardized conventional PCR assay. Hence a reliable test is required for the definite identification of *C. parapsilosis* complex.

Molecular techniques are always better choice in accurately identifying *Candida* species especially *C. parapsilosis* complex which can be performed from culture or directly from the samples requiring less time compared to phenotyping methods. DNA extraction, PCR amplification followed by restriction fragment length polymorphism (PCR-RFLP), DNA sequencing, MALDI-TOF-MS studies, and real-time PCR experiments involving probe primers are among the several molecular techniques that have been developed for identification. However, these methods involve costly reagents or equipment, and they are time-consuming, tedious, and technically challenging. Therefore, a PCR done using a specific primer will further reduce the time required for confirmation of the species.

This conventional PCR by in-house designed complex specific primers accurately detected all *Candida parapsilosis* complex (n=58) which correlates with the gene sequencing results, thereby validating the PCR specificity. In addition, other *Candida* species were also tested to ascertain the robustness of the PCR assay. Hence in this study by using complex specific primers, the PCR shows 100% sensitivity and specificity for *C. parapsilosis* complex isolates.

CONCLUSION:

This conventional PCR assay is a cost-effective method which can be used in standalone lab where advanced molecular techniques are not available for *C. parapsilosis* complex identification. Since it is done directly from clinical isolates it provides quick results, allowing for timely treatment and management of infection in high-risk patients and catheter related infections where its prevalence is higher. Accurate identification of *C. parapsilosis* complex facilitates early detection of outbreaks and monitoring of antifungal resistance patterns enabling prompt implementation of infection control measures. It facilitates epidemiological investigations in various geographic location and aids in determining the prevalence of *C. parapsilosis* complex and identify its risk factors.

FIGURES

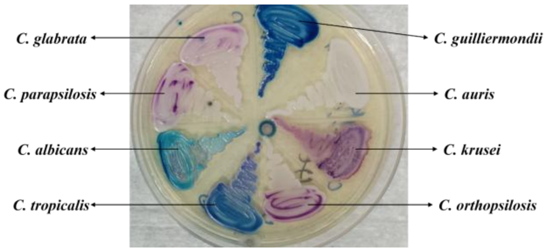

Fig. 1. Phenotypic identification of different *Candida* species on Hichrome agar

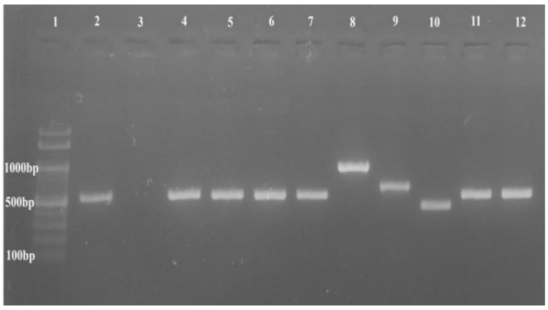

Fig. 2. PCR amplification of ITS region for *Candida* species using PAN fungal primers.

Lane 1: DNA marker (100bp), Lane 2: Positive control (ATCC *C. albicans* 90028), Lane 3: Negative control (Nuclease free water), Lane 4: *C. albicans*, Lane 5: *C. tropicalis*, Lane 6: *C. parapsilosis*, Lane 7: *C. krusei*, Lane 8: *C. glabrata*, Lane 9: *C. guilliermondii,* Lane 10: *C. auris*, Lane 11: *C. orthopsilosis*, Lane 12: *C. metapsilosis*

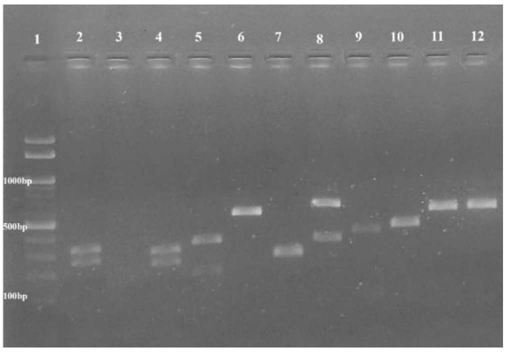

Fig. 3. PCR-RFLP of different *Candida* species using *Msp*I restriction enzyme.

Lane 1: DNA marker (100bp), Lane 2: Positive control (ATCC *C. albicans* 90028), Lane 3: Negative control (Nuclease free water), Lane 4: *C. albicans*, Lane 5: *C. tropicalis*, Lane 6: *C. parapsilosis*, Lane 7: *C. krusei*, Lane 8: *C. glabrata*, Lane 9: *C. guilliermondii,* Lane 10: *C. auris*, Lane 11: *C. orthopsilosis*, Lane 12: *C. metapsilosis*

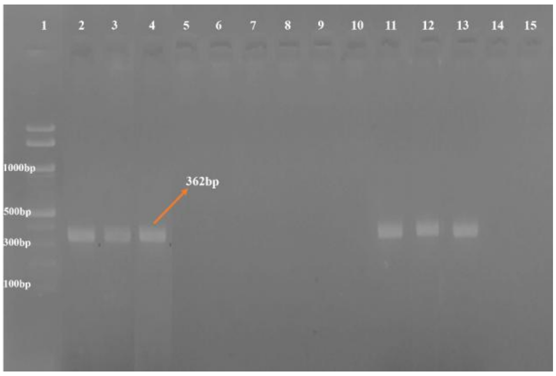

Fig. 4. Amplification of PCR products using complex specific primers with control strains.

Lane 1: DNA marker (100bp), Lane 2: Positive control (ATCC *C. parapsilosis* 90018), Lane 3: Positive control (sequence confirmed *C. orthopsilosis*), Lane 4: Positive control (sequence confirmed *C. metapsilosis*), Negative control (ATCC *C. albicans* 90028), Lane 6: Negative control (ATCC *C. tropicalis* 750), Lane 7: Negative control (*C. krusei*), Lane 8: Negative control (*C. glabrata*), Lane 9: Negative control (*C. guilliermondii*), Lane 10: Negative control (*C. auris*), Lane 11: Sample-*C. parapsilosis* complex, Lane 12: Sample-*C. parapsilosis* complex, Lane 13: Sample-*C. parapsilosis* complex, Lane 14: Sample-other *Candida* species , Lane 13: Sample-other *Candida* species.

ACKNOWLEDGEMENTS:

None.

REFERENCES:

1. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J, *et al. Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev* 2012; *36*(2): 288-305.
2. Badali H, Rezaie S, Meis JF, Afshari SAK, Modiri M, Hagen F, *et al*. Microsatellite genotyping of clinical *Candida parapsilosis* isolates. *Curr Med Mycol* 2017; 3(4): 15-20.
3. Tavanti A, Davidson AD, Gow NAR, Maiden MCJ, 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.
4. Trofa D, Gácser A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. *Clin Microbiol Rev* 2008; 21(4): 606-625.
5. Govrins M, Lass-Flörl C. *Candida parapsilosis* complex in the clinical setting. *Nat Rev Microbiol* 2024; 22: 46-59.
6. Neji S, Hadrich I, Trabelsi H, Abbes S, Cheikhrouhou F, Sellami H, *et al*. Virulence factors, antifungal susceptibility and molecular mechanisms of azole resistance among *Candida parapsilosis* complex isolates recovered from clinical specimens. *J Biomed Sci* 2017; 24: 67.
7. de Toro M, Torres MJ, Maite R, Aznar J. Characterization of *Candida parapsilosis* complex isolates. *Clin Microbiol Infect* 2011; *17*(3): 418-424.
8. Kuhn DM, Mukherjee PK, Clark TA, Pujol C, Chandra J, Hajjeh RA, *et al.* 2004. *Candida parapsilosis* characterization in an outbreak setting. *Emerg Infect Dis* 2004; 10(6): 1074-1081.
9. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. *Nippon Ishinkin Gakkai Zasshi* 2006; 47(3): 225-229.
10. Vijayakumar R, Giri S, Kindo AJ. Molecular species identification of *Candida* from blood samples of intensive care unit patients by polymerase chain reaction–restricted fragment length polymorphism. *J Lab Physicians* 2012; 4(1): 1-4.
11. Maria S, Barnwal G, Kumar A, Mohan K, Vinod V, Varghese A, *et al*. Species distribution and antifungal susceptibility among clinical isolates of *Candida parapsilosis* complex from India. *Rev Iberoam Micol* 2018; 35(3): 147-150.
12. Arastehfar A, Daneshnia F, Najafzadeh MJ, Hagen F, Mahmoudi S, Salehi M, *et al*. Evaluation of molecular epidemiology, clinical characteristics, antifungal susceptibility profiles, and molecular mechanisms of antifungal resistance of Iranian *Candida parapsilosis* species complex blood isolates. *Front Cell Infect Microbiol* 2020; 10: 206.
13. Cantón E, Pemán J, Quindós G, Eraso E, Miranda-Zapico I, Álvarez M, *et al*. Prospective multicenter study of the epidemiology, molecular identification, and antifungal susceptibility of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* isolated from patients with candidemia. *Antimicrob Agents Chemo* 2011; 55(12): 5590-5596.
14. Xiao M, Fan X, Chen S, Wang H, Sun Z, Liao K, *et al*. Antifungal susceptibilities of *Candida glabrata* species complex, *Candida krusei*, *Candida parapsilosis* species complex and *Candida tropicalis* causing invasive candidiasis in China: 3 year national surveillance. *J Antimicrob Chemother* 2015; 70(3): 802-810.
15. Marinho SA, Teixeira AB, Santos OS, Cazanova RF, Ferreira CAS, Cherubini K, *et al*. Identification of *Candida* spp. by phenotypic tests and PCR. *Braz J Microbiol* 2010; 41(2): 286-294.
16. Farooqi J, Jabeen K, Saeed N, Zafar A, Brandt ME, Hasan R. Species identification of invasive yeasts including *Candida* in Pakistan: limitations of phenotypic methods. *J Pak Med Assoc* 2012; 62(10): 995-998.
17. Cendejas-Bueno E, Gomez-Lopez A, Mellado E, Rodriguez-Tudela JL, Cuenca-Estrella M. Identification of pathogenic rare yeast species in clinical samples: comparison between phenotypical and molecular methods. *J Clin Microbiol* 2010; 48(5): 1895-1899.
18. Latouche GN, Daniel HM, Lee OC, Mitchell TG, Sorrell TC, Meyer W. Comparison of use of phenotypic and genotypic characteristics for identification of species of the anamorph genus *Candida* and related teleomorph yeast species. *J Clin Microbiol* 1997; 35(12): 3171-3180.
19. Coignard C, Hurst SF, Benjamin LE, Brandt ME, Warnock DW, Morrison CJ. Resolution of discrepant results for *Candida* species identification by using DNA probes. *J Clin Microbiol* 2004; 42(2): 858-861.
20. Cordeiro RA, Sales JA, de Ponte YB, Mende PBL, Serpa R, Evangelista AJ, *et al*. Phenotype-driven strategies for screening *Candida parapsilosis* complex for molecular identification. *Braz J Microbiol* 2018; 49(1): 193-198.