Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Identification of *Candida* species from blood samples of neonates by PCR-RFLP method in Western U.P

Gunjan Dutta[1]*, Kamya Verma[1], Kanwaljit Kaur[2], Rajesh Bareja[3]

[1]*Dept. of Microbiology, Saraswathi Institute of Medical Sciences, Hapur, Uttar Pradesh, India*
[2]*Dept. of Pathology, Ajay Sangal Institute of Medical Sciences and Research and Aayushman Hospital, Shamli, Uttar Pradesh, India*
[3]*Dept. of Microbiology, World College Of Medical Sciences & Research And Hospital (WCMSRH), Jhajjar, Haryana, India*

ARTICLE INFO

Article history:
Received 14-05-2024
Accepted 12-07-2024
Available online 27-09-2024

Keywords:
Neonates
Candida
Candidemia
PCR-RFLP

ABSTRACT

Introduction: Correct identification of Candida species is necessary for actual therapy and epidemiology study. *Candida* species is the most frequent emerging cause of fungal infections globally. The most common cause of Candidemia worldwide is *Candida* albicans, however, non-*Candida albicans* (NCA) species like *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei and C. guilleirmondii* being reported

Aim and Objective: To identify the different species of *Candida* from blood specimen of neonates by PCR-RFLP technique.

Materials and Methods : Total 27 *Candida* isolates were collected from blood samples of neonates. All the clinical specimens were inoculated for the isolation of *Candida* species using standard mycological techniques. The isolates were cultured on Sabouraud's Dextrose Agar (SDA) and further, they were identified by PCR and RFLP method.

Result: Molecular methods like PCR-RFLP were used in identifying six important *Candida* species (*C. tropicalis, C. albicans, C. parapsilosis, C. glabrata, C.krusei and C.guilliermondii*) from blood. By using universal primers ITS-1 and ITS-4, PCR assay successfully amplified the ITS-1 and ITS-2 region of 27 isolates and the amplicon size of approximately 510-870 bp. This method is speedy, easy, and affordable which can be used in routine laboratory diagnostics for the quick isolation of Candida species from blood samples.

Conclusion: It is helpful for clinicians to start actual therapy in Candidemia patients before antifungal sensitivity results are obtainable.

1. Introduction

Candida species is the most frequent emerging cause of fungal infections globally.[1] It is also the fourth most significant cause of blood stream infection (BSI) in the United States. Due to various multi-centric studies, the picture is not clear in India. It has been also seen that Candidemia is also linked with an elevated mortality rate, which ranges from 10-49%.[2] The most general cause of worldwide Candidemia is *Candida albicans*, but it has been observed that non *Candida albicans* (NCA) species like *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei and C. guilleirmondii* are additionally involved.[2]

The most frequent species of *Candida* has been isolated from blood in India is *C.tropicalis*.[3] It has been observed from fewer studies that, fluconazole and other triazoles are resistant to *Candida* species are mostly *C. glabrata* and *C. krusei* whereas *C. parapsilosis* have been found susceptible to azole group of drugs.[4] A few studies have also shown

* *Corresponding author*.
E-mail address: gundutta@gmail.com (G. Dutta).

https://doi.org/10.18231/j.ijmmtd.2024.041

resistance to Amphotericin B and echinocandins.[5,6]

Phenotypic methods used for the isolation of *Candida* species are sugar assimilation, sugar fermentation, Dalmou plate technique, culture characteristics on SDA or CHROM agar etc, but these methods require 48-72 hrs or even more for the identification. Quick identification and detection of Candida species is necessary for the suitable treatment and protection of patients who suffer from Candidemia.[7]

We used molecular methods like PCR-RFLP which are more sensitive, quick, smooth and price- effective for the diagnosis of different species of *Candida* from blood isolates of neonates.

2. Materials and Methods

This was a prospective cross-sectional study for which ethical clearance was taken.

Single *Candida* isolate per patient's specimen was comprised in the study to avoid duplication of isolates. Mixed cultures were excluded from the study. In Table1, six standard strains used in the study with the sizes of ITS1-ITS4 PCR products for Candida species before and after digestion with MspI restriction enzyme are listed, which were provided by PGI Chandigarh.

2.1. Clinical isolates

Entirely 27 successive blood isolates were collected of neonates from the Neonatal Intensive Care Unit (NICU) for two years period at the Microbiology laboratory, Subharti University, Meerut.

All the clinical specimens were cultivated for the isolation of *Candida* species using standard mycological techniques. The isolates were cultured on Sabouraud's Dextrose Agar (SDA) and further, they were used for molecular examination.

2.2. DNA isolation

By using a True-prep DNA extraction kit (Molbio Diagnostics Pvt Ltd) all the isolates was extracted for DNA. The eluted DNA was stored at -20°C until use. To confirm the presence of DNA, the gel electrophoresis was done.

2.3. PCR assay

Amplification for PCR was perform in a final volume of 100 μl. Every reaction consists of 1 μl of template DNA, each forward (ITS1, 5'-TCC GTA GGT GAA CCT GCG G-3'and reverse (ITS4, 5'TCC TCC GCT TAT TGA TAT GC-3') primer at 0.2 μM, each deoxynucleoside triphosphate (dNTP) at 0.1 mM, 10 μl of 10X PCR buffer, and 2.5 U of Taq DNA polymerase. The amplification was pursued by 25 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 45 s, extension at 72°C for 1 min, with a final extension step of 72°C for 7 minutes. 1.5 % (w/v) agarose gel electrophoresis in TAE buffer were used to visualize the amplified products and then they stain with ethidium bromide under UV light and photographed.

2.4. RFLP analysis and gel electrophoresis

To perform the digestion, a 20μl aliquot of PCR product was incubated with 10 U of MspI in a final reaction volume of 25μl for 2hr at 37°C. By 1.8% agarose gel electrophoresis in TAE buffer for approximately 45 min at 100V restriction fragments were separated and visualized by staining with ethidium bromide.

3. Results

By using universal primers ITS-1 and ITS-4, PCR assay successfully amplified the ITS-1 and ITS-2 region of 27 isolates and the amplicon size of approximately 510-870 bp (Figure 1) was obtained from medically important *Candida* species (C. *albicans, C. tropicalis, C. glabrata, C. parapsilosis, C.krusei and C.guilliermondii*). The restriction enzyme Msp I was used for the RFLP method in one study which was performed in Iran.[8](Figure 2).

Digestion of ITS region by Msp I give rise to two bands in each species except for *C. parapsilosis* as it does not have any specific restriction site for Msp I. The pattern for bands was different for each *Candida* species and hence it was simple to differentiate them. All strains were compared with the control strains as shown in the table (Table 1). Dispersal of *Candida* species isolated from blood of NICU patients by PCR-RFLP are mentioned in (Table 2).

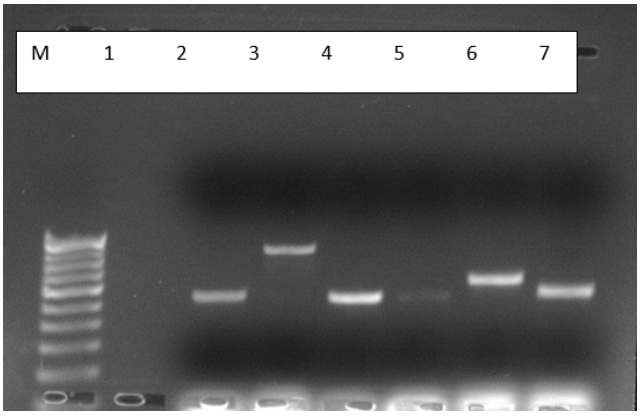

Figure 1: PCR products from *Candida* species. Lane 1- NC (Negative Control), Lanes 2- ATCC C.albicans, Lane 3-ATCC *C.glabrata*, Lane 4- ATCC C.krusei, Lane 5-ATCC *C.tropicalis*, Lane 6-ATCC *C.guilliermondii*, Lane 7: *C.parapsilosis*. Lane M: 100bp Molecular size marker

4. Discussion

For the handling of Candidemia and to lower the death rate related with it, early diagnosis of *Candida* from

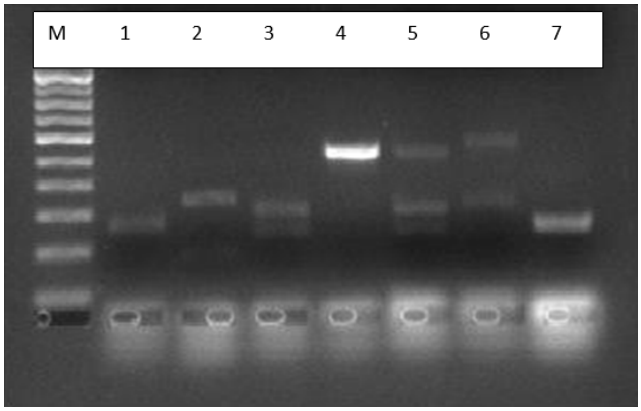

Figure 2: *Candida* strains with the enzyme MspI. Lanes 1 and7: C.krusei, Lane 2: *C. tropicalis*, Lane 3: C.albicans Lane 4: *C.parapsilosis*, Lane 5: C guilliermondii, Lane 6: *C.glabrata*, Lane M: 100bp Molecular size marker

Table 1: Sizes of ITS1-ITS4 PCR products for *Candida* species before and after digestion with MspI restriction enzyme.

***Candida* species**	**Size of ITS1-ITS4**	**Size of restriction product**
C.tropicalis	524	340,184
C. albicans	535	297,238
C. parapsilosis	520	520
C.krusei	510	261,249
C.glabrata	871	557,314
C.guilliermondii	608	371,155,82

Table 2: Distribution of *Candida* species isolated from blood of NICU patients by PCR-RFLP.

***Candida* species**	**Number (%)**
C.tropicalis	14
C. parapsilosis	06
C. albicans	04
C.glabrata	01
C.krusei	01
C.guilliermondii	01
Total	27

blood stream infections are very important. Currently to diagnose *Candida* up to species level by using phenotypic methods will take 48 to 72 hrs. Because of restrictions of conventional techniques, genotypic method mainly PCR is being operated for untimely detection of *Candida* from blood.

For the speedy detection of *Candida* species, many molecular methods such as DNA sequencing, RAPD (Random Amplified Polymorphic DNA), real time PCR etc. have been developed, but these methods require skilled workers and are more expensive.[9]

In our study, PCR-RFLP was performed for 27 *Candida* samples from the blood of neonatal intensive care unit. Universal primers, ITS1 and ITS2 were able to amplify the ITS region of genomic DNA by PCR. RFLP was done for the amplified products with the help of MSP I restriction enzyme and all the isolates were correctly recognized up to the species level. Six *Candida* species were detected from NICU patient's blood samples *(C. tropicalis, C.parapsilosis, C.albicans, C.glabrata, C.krusei and C.gullermondii*).

In our study, non *Candidaalbicans* (NCA) spp. were more commonly isolated than *Candida albicans*. The same finding also noticed in other studies done by Vaibhav Misra et.al.[10] and Asifa Nazir et.al.[11] In epidemiological studies indicated that *Candida* tropicalis is involved in about 67-90% cases of candidemia.[12] In our study also *C.tropicalis* was the prominent one among other NCA species.

It has been found that PCR-RFLP is a quick and authentic technique to speciate *Candida* isolates in several studies also. In one research, MSP I enzyme with PCR-RFLP assay used for detection of medically essential fungi.[13] Similar study from Iran, by Mirhendi et.al. also identified the six *Candida* isolates by PCR-RFLP.[8]

In other study of Iran, clinical isolates of *Candida* were identified by Real time PCR by High Resolution Melting Analysis (HRMA), and in one study of Vienna, various molecular methods are described for the diagnosis of Candida species.[14,15]

In our study, we found PCR-RFLP to be an easy, speedy and affordable technique for the isolation of *Candida* species from blood isolates.

The whole procedure of PCR-RFLP will finish in approximately seven hours whereas conventional methods require 48-72 hours to speciate the isolates. This will help to start the actual therapy of Candidemia by clinicians before antifungal sensitivity reports are obtainable. For example *C.glabrata* is usually resistant to fluconazole, but it is safe and effective in invasive candidiasis treatment in infants.[16] In one study, randomized controlled trials were done and based on that data the recommended antifungal were echinocandins as first line agent in older children and adults for Candidemia and disseminated candidiasis.[17,18] Therefore, Candidemia sufferers can be treated by Amphotericin B and echinocandins.

5. Conclusion

PCR-RFLP has an advantage of high discrimination power, potential for wide range of speciation of *Candida* species and can be used for quality control of our phenotypic methods when these phenotypic methods fail to identify the species. It is also helpful for clinicians to start actual therapy in Candidemia patients before antifungal sensitivity reports are obtainable.

6. Ethical Approval

This study was conducted under Ethical committee certificate number-SMC/EC/2014/47.

7. Conflict of interest

There was no conflict of interest.

8. Source of Funding

None.

References

1. Dutta G, Pandey A. Pcr-rflp assay identification of non-albicans candida species by mspi restriction enzyme. *Indian J Appl Res*. 2020;10(7). doi:10.36106/ijar.
2. Pfaller MA, Diekema DJ. Epidemiology of Invasive Candidiasis: a Persistent Public Health Problem. *Clin Microbiol Rev*. 2007;20(1):133–63.
3. Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemiain a tertiary care centre of north India: 5-year study. *Infection*. 2007;35(4):256–9.
4. Sahni V, Agarwal SK, Singh NP, Anuradha S, Sikdar S, Wadhwa A, et al. Candidemia - An Under-recognized Nosocomial Infection in Indian Hospitals. *J Assoc Physicians India*. 2005;53:607–11.
5. Pfaller MA, Diekema DJ. International Fungal Surveillance Participant Group. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of Candida. *Clin Microbiol Infect*. 2004;10(1):11–23.
6. Nolte FS, Parkinson T, Falconer DJ, Dix S, Williams J, Gilmore C, et al. Isolation and characterization of fluconazole and amphotericin B-resistant Candida albicans from blood of two patients with leukemia. *Antimicrob Agents Chemother*. 1997;41(1):196–9.
7. Krogh-Madsen M, Arendrup MC, Heslet L, Knudsen JD. Amphotericin B and caspofungin resistance in Candida glabrata isolates recovered from a critically ill patient. *Clin Infect Dis*. 2006;42(7):938–44.
8. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A One-Enzyme PCR-RFLP assay for identification of six medically important Candida species. *Nihon Ishinkin Gakkai Zassh*. 2006;47(3):225–9.
9. Vijaykumar 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.
10. Vaibhav M, Himanshi B, Sanjay SK. Incidence of neonatal fungaemia in a tertiary care hospital in north india. *J Evid Based Med Healthc*. 2016;3(47):2326–31.
11. Asifa N. Non albicans Candida in Neonatal Septicemia- An emerging clinical entity. *Int J Biomed Res*. 2016;7(2):47–50.
12. Manoj K, Ravindra KB, P A. Candida Species Isolated From Blood Culture in Neonatal Septicemia Patients Admitted at RIMS, Ranchi. *Int J Med Res Prof*. 2018;4(1):622–5.
13. Dutta G. Identification of Candida species by phenotypic and PCR-RFLP assay. *Int J Scientific Res*. 2020;9(8). doi:10.36106/ijsr.
14. Nejad EE, 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*. 2022;34(10):e23444. doi:10.1002/jcla.23444.
15. Iris C, Spettel K, Willinger B. Molecular Methods for the Diagnosis of Invasive Candidiasis. *J Fungi*. 2020;6(3):101. doi:10.3390/jof6030101.
16. Rachel G, Dniel KB. Neonatal candidiasis: Diagnosis, prevention, and treatment. *J Infect*. 2014;69(1):19–22.
17. Pappas PG, Kauffman CA, Andes D, Benjamin DK, Calandra TF, Edwards JE, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2009;48(5):503–35.
18. Schwarze R, Penk A, Pittrow L. Treatment of candidal infections with fluconazole in neonates and infants. *Eur J Med Res*. 2000;5(5):203–8.

Author biography

Gunjan Dutta, Associate Professor

Kamya Verma, Associate Professor

Kanwaljit Kaur, Associate Professor

Rajesh Bareja, Professor https://orcid.org/0000-0002-1240-0904

Cite this article: Dutta G, Verma K, Kaur K, Bareja R. Identification of *Candida* species from blood samples of neonates by PCR-RFLP method in Western U.P. *IP Int J Med Microbiol Trop Dis* 2024;10(3):236-239.