E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/16.01.111

Research Article

Detection of Virulence Factors of Candida Species Isolated From Neonatal Candidemia at Tertiary Care Hospital in North India

Dr. Baby[1*], Dr. Perbhat Kansal[2]
[1*]Associate professor, Department of Microbiology, Dr. S.S. Tantia MCH&R Sriganganagar Rajasthan.
[2]Associate professor, Department of Pharmacology, Dr. S.S. Tantia MCH&R Sriganganagar Rajasthan.
Email: [1*]docmittal89@gmail.com, [2]perbhat.kansal@gmail.com
Corresponding author: Dr. Baby
Email: docmittal89@gmail.com
Associate professor, Department of Microbiology, Dr. S.S. Tantia MCH&R Sriganganagar Rajasthan.
Postal address: A-14, Sky Hi Residency, 2 ML, Sri Ganganagar, Rajasthan-335001.
Received: 11.01.26, Revised: 16.02.26, Accepted: 14.03.26

ABSTRACT
Background: Fungus being ubiquitous, but infections caused by it are rare and as a suspect for sepsis are rarest. So, it becomes a cause for higher morbidity and mortality especially in state of immune-compromised state like neonates admitted in NICU. Hence to treat it appropriately, we must know its emergence, virulence and trend of it. So we design this study in neonates, one of most vulnerable group to study its virulence and effects on them.
Method: It is a prospective study conducted for one and half years on 44 positive neonates detected for candidemia. Using most advanced and also the conventional methods we differentiate the species and also studied in detail about their virulence and end results on patients
Results: Incidence is 10.33%, with dominance of non albicans candidemia (65.9%) over *C. albicans* septicaemia. Virulence is also higher in non albicans Candida (NAC) as compared to *C. albicans*. The mortality (34%) detected was higher in NAC but average duration of hospital stay (14.5) is longer in *C. albicans* due to its more resistance towards anti- fungals.
Conclusion: In this long study on neonatal septicaemia, we found that NAC were emerging pathogens and were more virulent than *C. albicans*

Keywords: Candida, Non-Albicans, Neonates, Sepsis, Virulence Factor.

INTRODUCTION

Fungus being present widespread in environment is usually non-pathogenic and innoxious in nature, as some of them are normal flora that is residing on our skin, mucosal surfaces, vagina, gastrointestinal and urinary tract.[1] The medical field is advancing rapidly but, even then the neonatal sepsis especially related to fungal infections is in poor focus, causing many deaths in neonates. It also has been noted that candidemia incidence is increasing from last two decades in the world. It is usually related to increase in virulence or increase in resistance or both in isolated Candida species. [2,3]

In neonates as the normal flora is not yet developed, so the Candida infection becomes more of inoculated than opportunistic one, already been proved in various studies that first step of invasive candidiasis was skin and GIT inoculation.[4] So in this article we are going to focus on septicaemia in neonates due to Candida spp. especially main attention on the virulence of such species.

In neonates, Candida spp. is a major cause for late neonatal sepsis as it is listed as third most common organism to be found.[5] We found that neonates are more vulnerable than adults or children for Candidemia, so determining the virulence and susceptibility comes to major focus for treating such infections in delicate age group where specific and nonspecific immune functions are immature hence poor.

Furthermore, increase in use of antibiotics and presence of various other risk factors predispose neonates more towards blood stream infection (BSI) than that of local mucosal infections. [6,7] Traditionally *C. albicans* was predominantly found species in clinical specimens but now a days there is increase in trend of isolation of NAC species from various infectious conditions. [8,9]

Transition of a microorganism from colonizer to pathogen depends upon various virulence factors. So, crucial virulence factors responsible for infective nature of Candida spp. include adhesion to cells, biofilm formation, proteinase production, pseudo

hyphae formation, production of phospholipase, phenotypic switching etc. [10] Hence we designed this study in such a way that we can correlate virulence factors with severity of clinical symptoms and outcome for the treatment.

Biofilm is structural community that is formed of aggregation of microbial cells and exo-polysaccharide structures. Biofilm formation by Candida spp. results in avoidance of protective mechanism by host and promote as long-lasting source of infection, that may result in development of antimicrobial resistance and treatment failure.[11]

Aspartyl proteinases are degrading enzymes that are secreted by pathogenic spp. of Candida. Secreted proteinases are considered to be contributing factor for tissue damage, adhesion to cells and attacking the host immune response. Similarly, phospholipase is a heterogeneous group of enzymes that causes hydrolysis, ultimately affecting the integrity of membrane and results in cell lysis. [11]

From various evidences we also found that emergence of various virulence factors in Candida species over the time also cause the resistance to most commonly used anti-fungals that the azoles.[12]

As we further see that resistance is in the main focus and is hindrance in successful treatment of patients, so this article itself focus on the virulence factors their emergence and their distribution among various Candida spp. causing neonatal candidemia.

MATERIALS AND METHODS

Study design and setting: This prospective observational study was carried out in a tertiary care teaching hospital located in North India after obtaining approval from the Institutional Ethics Committee. Neonates admitted to the Neonatal Intensive Care Unit (NICU) over a period of one and a half years were screened for inclusion in the study.

Study Population: Neonates with clinical features suggestive of sepsis and fulfilling the predefined inclusion criteria were enrolled. Written informed consent was obtained from parents or legal guardians prior to enrolment. During the study period, a total of 426 neonates were included. Demographic and clinical data were recorded and systematically entered into a Microsoft® Excel spreadsheet for analysis. Only cases showing pure growth of *Candida* species in blood culture, confirming candidemia, were considered for further evaluation [9].

Blood Culture and Identification of Candida Species: Blood samples were collected under strict aseptic precautions and processed using the BACTEC 9120 automated blood culture system in accordance with standard laboratory protocols [13]. Samples flagged positive were subjected to Gram staining, followed by subculture on blood agar and Sabouraud's dextrose agar (SDA). Presumptive identification was performed based on colony morphology and microscopic appearance. Species-level identification was carried out using germ tube test, chlamydospore formation, carbohydrate fermentation and assimilation tests and was further confirmed using the VITEK-2 automated identification system [14,15].

Detection of Virulence Factors

A. Biofilm Formation: Biofilm production was assessed using both visual observation and spectrophotometric microtiter plate methods. Sabouraud's dextrose broth (SDB) was prepared as per manufacturer's instructions. A saline suspension of 24-hour-old yeast culture was prepared and turbidity adjusted to approximately 3×10^7 CFU/mL. Each well of a sterile microtiter plate was inoculated with 20 µL of yeast suspension and 180 µL of SDB, followed by incubation at 35°C for 24 hours. After incubation, wells were gently washed to remove planktonic cells. Optical density was measured at 405 nm after adding 200 µL of distilled water. Biofilm formation was graded based on percentage transmittance blockage (%Tbloc) as follows: Negative: <5**,** 1+: 5–20**,** 2+: 20–35**,** 3+: 35–50**,** 4+: ≥50[16].

B. Proteinase Estimation: Proteinase production was evaluated using bovine serum albumin (BSA) agar. A 1% (w/v) BSA solution was prepared, filter sterilized, and added to molten Brain Heart Infusion agar (5% w/v) at 45°C. A loopful of yeast culture was inoculated at the center of each agar plate and incubated at 37°C. Plates were examined daily for up to 10 days. Following incubation, plates were stained with 0.1% amido black dye to visualize proteolytic activity. Proteinase activity was graded as:

- (–): No visible halo
- (+): Zone of proteolysis measuring 1–2 mm around the colony

- (++): Zone of proteolysis exceeding 2 mm beyond the colony margin [17]

C. Phospholipase Estimation: Phospholipase activity was assessed using egg yolk agar medium. A saline suspension of yeast cells was prepared and inoculated onto the surface of egg yolk agar plates, followed by incubation at 37°C for 48 hours. After incubation, the diameter of the colony and the surrounding zone of precipitation were measured. Phospholipase activity was expressed as the Pz value, calculated as the ratio of colony diameter to the total diameter of the colony plus precipitation zone. A Pz value <1 indicated phospholipase production, while a Pz value <0.7 suggested higher virulence [18].

RESULTS

During the study period, a total of 426 blood culture samples were received from neonates with suspected septicaemia admitted to the Neonatal Intensive Care Unit (NICU). Out of these, 44 isolates (10.33%) showed pure growth of Candida species, confirming candidemia.

Species Distribution: Species identification was performed using germ tube test, corn meal agar morphology, carbohydrate fermentation and assimilation tests, and automated identification by VITEK-2 system. Of the 44 Candida isolates, Candida albicans accounted for 15 (34.1%) isolates, while non-albicans Candida (NAC) species constituted 29 (65.9%) isolates. Among the NAC isolates, Candida tropicalis was the most frequently isolated species (12/29), followed by Candida pelliculosa (10/29) and Candida krusei (7/29) (Table I).

Table I : Distribution of Non albicans Candida (NAC) spp.

Specie name	Number
C. tropicalis	12 (27.3%)
C. pelliculosa	10 (22.7%)
C. krusei	07 (15.9%)
Total	29 (65.9%)

Mode of Delivery and Epidemiological Characteristics

Among neonates with candidemia, 62.5% were delivered by normal vaginal delivery (NVD), whereas 37.5% were delivered by lower segment caesarean section (LSCS). Of the neonates delivered by NVD, 97% were institutional deliveries, while 3% were home deliveries with a history of referral from other healthcare centres, suggesting possible healthcare-associated acquisition.

Virulence Factor Expression

Among the 44 *Candida* isolates, biofilm formation was observed in 27 isolates (61.3%), proteinase production in 20 isolates (45.5%), and phospholipase activity in 9 isolates (20.4%) (Table II).

Table II: Frequency of Virulence factors among various Candida spp.

Candida species	Biofilm n (%)	Proteinase n (%)	Phospholipase n (%)
C. albicans (n=15)	9 (60.0)	8 (53.3)	6 (40.0)
C. tropicalis (n=12)	7 (58.3)	5 (41.7)	2 (16.7)
C. pelliculosa (n=10)	6 (60.0)	4 (40.0)	0 (0)
C. krusei (n=7)	5 (71.4)	3 (42.9)	1 (14.3)
Total (n=44)	27 (61.3)	20 (45.5)	9 (20.4)

Biofilm Formation: Among *C. albicans*, 9 out of 15 isolates (60%) demonstrated biofilm production. The majority produced weak to moderate biofilm (1+ or 2+), while only one isolate (11%) showed strong biofilm production (3+). In comparison, 18 NAC isolates were biofilm producers, of which 3 isolates (16%) demonstrated strong biofilm formation (3+), indicating a higher proportion of strong biofilm producers among NAC species.

Proteinase Activity: Proteinase production was detected in 20 of the 44 isolates. Among these, NAC species accounted for 12 isolates, with *C. tropicalis* contributing the highest proportion (41%), followed by *C. pelliculosa*.

Phospholipase Activity: Phospholipase production was detected in 9 isolates, of which *C. albicans* constituted the majority (n = 6), followed by *C. tropicalis*. No phospholipase activity was detected in *C. pelliculosa* isolates.

E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/16.01.111

Combined Virulence Factor Expression: Only one isolate of *C. albicans* demonstrated production of all three virulence factors (biofilm, proteinase, and phospholipase). None of the NAC isolates produced all three factors simultaneously. However, co-expression of any two virulence factors was observed more frequently among NAC isolates (9 isolates) compared to *C. albicans* (3 isolates). (Fig 1)

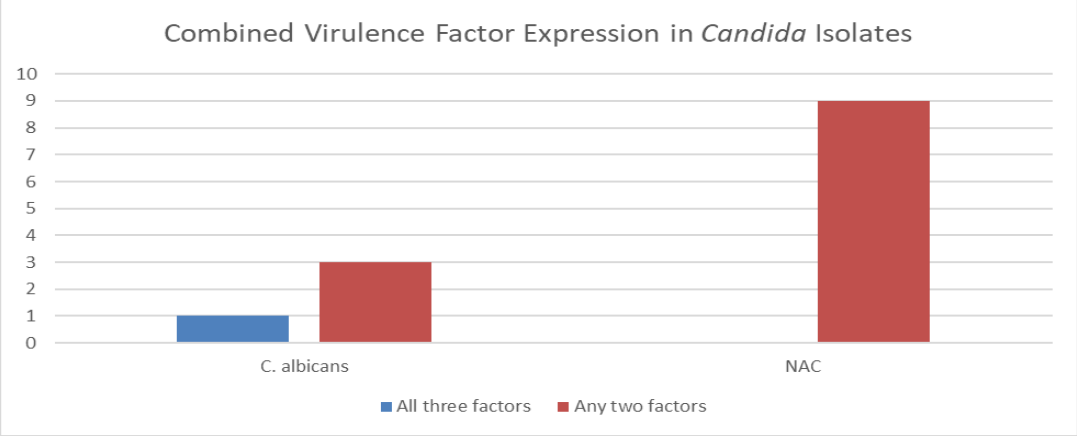

Fig 1- Combined Virulence Factor Expression in Candida Isolates

Duration of Hospital Stay: The mean duration of hospital stay among neonates with candidemia was 12.8 days, ranging from 4 to 37 days. The mean duration of stay was longer in neonates infected with NAC species (14.5 days) compared to those infected with C. albicans (11.9 days).

Disease Severity and Supportive Care: Requirement for mechanical ventilation was observed exclusively among neonates infected with NAC species. Among these, C. krusei was associated with the highest proportion of severe disease, with 4 out of 7 cases requiring intensive respiratory support.

Maternal and Perinatal Risk Factors: After excluding other high-risk indications, maternal and perinatal factors observed among candidemia cases included premature rupture of membranes (n = 4), prolonged labour (n = 2), intrapartum antibiotic administration (n = 13), and delivery by LSCS (n = 12). The association of these factors with neonatal candidemia could not be clearly established in the present study.

Mortality and Outcome: Out of the 44 neonates with candidemia, 15 cases (34%) resulted in mortality. Among these deaths, 4 cases (26%) were associated with C. albicans, while 11 cases (74%) were attributed to NAC species. Species-wise mortality was highest with C. krusei (4/7), followed by C. tropicalis (5/12) and C. pelliculosa (2/10). (Table -III)

Table – III Outcome and Mortality According to *Candida* Species

Candida species	Total cases	Deaths n (%)	Discharged n (%)
C. albicans	15	4 (26.7)	10 (66.7)
C. tropicalis	12	5 (41.7)	6 (50.0)
C. pelliculosa	10	2 (20.0)	5 (50.0)
C. krusei	7	4 (57.1)	2 (28.6)

Discharge Outcome: Discharge in satisfactory condition was observed in 10 out of 15 neonates (66.7%) infected with *C. albicans*, compared to 13 out of 29 neonates (44.8%) infected with NAC species.

DISCUSSION

This study was designed to find out the virulence factors and their trends among various species of Candida. The target population was most delicate one, the new born which don't have any active immunity component present in them.

Out of 426 suspected cases of septicaemia, 10.26% were found to be positive for candidemia, nearly similar to the other studies having incidence rate ranging from 9.8%-13%.[19,20] Overall results were in coherence to report published by WHO on sepsis.[21] But in some studies it was reported as higher as 20-58%[9,22,23] and as lower as 2-4%.[24,25] There is huge variability in data, which can be explained by multiple factors. First and most

important factor which we consider is use of different techniques to detect the organism, secondly the hygiene practices, thirdly and lastly awareness among treating physician.

With the use of Vitek-2 automation method we differentiate the 44 isolates into species of Candida. As it has been already proved that this system has accuracy rate nearly 98.5%,[26] so we can confidently say that we isolated *C. albicans*-15, *C. tropicalis*-12, *C. pelliculosa*-10 and *C. kruesi*-7. So in this study NAC were 65.9%, indicating increase in infection rate by NAC as compared to C. albicans. Our data was supported by various other studies in which it had already been proved that the pattern is shifting from C. albicans to NAC. [27-29] So it can be attributed to increase in use of fluconazole prophylaxis and also use of azoles in maintenance therapy. Some researchers also proved that due to increase in virulence more in NAC than *C. albicans* also cause increase in spread of NAC rapidly.[30] For such increase in NAC we can also say that local factors are responsible like low sterile practices, low weight births, repeated hospital exposure and multigravida in India.

Out of 426 received, 44 were positive for candidemia, as we focused on mode of delivery; we found 62.5% were NVD patients who turned out to be positive for candidemia. It is a well-known fact that Candida can be found as normal flora of vagina, so neonates may be getting affected during the delivery process. But as per the speciation concerned the NAC, it was never found to be normal in vaginal flora. Hence most of our neonates were affected post delivery process concluding nosocomial spread is there in our study. But a famous study on vertical transmission of Candida contradicts this finding as it strongly suggests the presence of vertical transmission is common than nosocomial.[31] There is once interesting study concluding transmission via NVD was 12.8% which was increased due to various risk factors like PROM, instrumentation and prolonged labour, but also suggesting evidence of nosocomial transmission same as per our study suggests.[32]

As we further analyse our results, we found that NAC were more pathogenic in nature than *C. albicans*. It is a well-known fact that pathogenicity of species is a result of strains, immune status of patient and local conditions of infection. As it a complex process so need to be evaluated at each stage.[33] Three well established factors which increase pathogenicity of Candida are biofilm formation, phospholipase and proteinase production.

Out of 44 isolates, 27 (61.3%) were found positive for biofilm formation; similar results were also reported by other studies. [34-36] It was also postulated that this factor is usually absent in commensal organism, hence a main differentiating feature. We also had an interesting finding, in our study this pathogenic feature was dominated by NAC, which was nearly equal in findings in study by Bhatt et. al and many others. [16,34,37] So biofilm production was most common and main pathogenic character of Candida. The fact that NAC were more emerging threat as of higher pathogenicity was also demonstrated by Hernandez *et al*. [38]

The second most important factor for pathogenesis was production of phospholipase by Candida. This phospholipase enzyme target cell membrane; phospholipids produce cell lysis and direct host damage.[11] In our study we found that 20% of the isolates were producing it, it varies widely in various studies conducted by various authors ranging 6%-44%. [11,39] These variations can be explained by species variation and sometimes different microbial methods of culture and observation. One interesting study exclusively designed for phospholipase activity indicates that almost every Candida spp. showed its presence but clinically important pathogenic nature was shown in 60% of the isolates.[40] Our data also suggested that Candida possessing such activity infecting the host ended in adverse outcomes.

In our study the third most important and widely spread pathogenic factor proteinase production was studied in details. As species exhibiting aspartyl proteinase were more pathogenic in nature, were also difficult to treat. In our study, maximum proteinase activity was observed in 58.33% isolates of C. tropicalis. It has been reported that there is a correlation between production of proteinase and virulence and the most virulent NAC spp. like *C. tropicalis* produces more proteinases in vitro than the less virulent spp. [41]. This observation was also noted in our study

In our case prolonged hospitalization was of average 17days. NAC (12/20) were dominantly demonstrating it, indicating the rising trends of NAC infection. There results were similar to a study by Deorukar et al. [39], but much higher rates were demonstrated by other study [11]

The mortality rate in this study reported was 34% which is much lower than the studies reported elsewhere. [42,43] It can be explained on the basis of good physician awareness, quality care and prompt treatment. In last we found that among NAC, *C. kruesi* was most virulent clinically, causing maximum deaths and demand prolonged mechanical ventilation support (n=4), similarly demonstrated in study by Sandhu *et al.* [43]

CONCLUSION

This study demonstrates a predominance of non-*albicans Candida* species in neonatal candidemia, with *Candida tropicalis* being the most frequently isolated species. Non-*albicans Candida* exhibited greater expression of virulence factors, particularly biofilm formation, and were associated with increased disease severity and higher mortality compared to *Candida albicans*. These findings highlight the clinical importance of early species identification, awareness of virulence potential, and timely initiation of appropriate antifungal therapy to improve outcomes in neonatal intensive care units.

Acknowledgements

Our sincere thanks to all the study participants. We acknowledge the support of everyone involved in this study.

Conflicts Of Interest, Financial Support and Sponsorship

None

REFERENCES

1. Odds FC. Pathogenic fungi in the 21st century. *Trends Microbiol*. 2000;8(5):200–201.
2. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev*. 2007;20(1):133–163.
3. Reda NM, Hassan RM, Salem ST, Yousef RHA. Prevalence and species distribution of Candida bloodstream infection in children and adults in two teaching university hospitals in Egypt: first report of Candida kefyr. *Infection*. 2023; 51:389–395.
4. De Rose DU, Santisi A, Ronchetti MP, Martini L, Serafini L, Betta P, et al. Invasive Candida infections in neonates after major surgery: current evidence and new directions. *Pathogens*. 2021;10(3):319.
5. Pammi M, Holland L, Butler G, Gácser A, Bliss JM. Candida parapsilosis is a significant neonatal pathogen: a systematic review and meta-analysis. *Pediatr Infect Dis J*. 2013;32(5):206–216.
6. Roilides E. Invasive candidiasis in neonates and children. *Early Hum Dev*. 2011; 87:75–76.
7. Benjamin DK Jr, Stoll BJ, Gantz MG, Walsh MC, Sánchez PJ, Das A, et al. Neonatal candidiasis: epidemiology, risk factors and clinical judgment. *Pediatrics*. 2010;126(4):865–873.
8. Gupta A, Gupta A, Varma A. Candida glabrata candidemia: an emerging threat in critically ill patients. *Indian J Crit Care Med*. 2015;19(3):151–154.
9. Juyal D, Sharma M, Pal S, Rathaur VK, Sharma N. Emergence of non-albicans Candida species in neonatal candidemia. *N Am J Med Sci*. 2013;5(9):541–545.
10. Ibrahim AS, Mirbod F, Filler SG, Banno Y, Cole GT, Kitajima Y, et al. Evidence implicating phospholipase as a virulence factor of Candida albicans. *Infect Immun*. 1995;63(5):1993–1998.
11. Mohandas V, Ballal M. Distribution of Candida species in different clinical samples and their virulence: biofilm formation, proteinase and phospholipase production. *J Glob Infect Dis*. 2011;3(1):4–8.
12. Li Y, Hind C, Furner-Pardoe J, Sutton JM, Rahman KM. Understanding the mechanisms of resistance to azole antifungals in Candida species. *JAC Antimicrob Resist*. 2025;7(3): dlad0xx.
13. BD Diagnostics. *BACTEC™ 9240/9120 System User's Manual*. Sparks (MD): BD; 2009.
14. Chakrabarti A, Shivaprakash MR. *Medical Mycology Laboratory Procedures*. Chandigarh: Styadeep Offset Printers; 2008. p. 54–68.
15. Melhem MSC, Bertoletti A, Lucca HRL, Silva RBO, Meneghin FA, Szeszs MW. Use of the VITEK 2 system to identify and test antifungal susceptibility of clinically relevant yeast species. *Braz J Microbiol*. 2013;44(4):1257–1266.
16. Shin JH, Kee SJ, Shin MG, Kim SH, Shin DH, Lee SK, et al. Biofilm production by isolates of Candida species recovered from non-neutropenic patients. *J Clin Microbiol*. 2002;40(4):1244–1248.
17. Lahkar V, Saikia L, Patgiri SJ, Nath R, Das PP. Estimation of biofilm, proteinase and phospholipase production of Candida

species isolated from HIV-infected patients. *Indian J Med Res*. 2017;145(5):635–640.

18. Erum R, Samad F, Khan A, Kazmi SU. Production of extracellular hydrolytic enzymes of Candida species and correlation with antifungal resistance. *BMC Microbiol*. 2020; 20:368.
19. Goel N, Ranjan PK, Aggarwal R, Chaudhary U, Sanjeev N. Emergence of non-albicans Candida in neonatal septicemia and antifungal susceptibility. *J Lab Physicians*. 2009;1(2):53–55.
20. Benjamin DK Jr, Stoll BJ, Fanaroff AA, McDonald SA, Oh W, Higgins RD, et al. Neonatal candidiasis among extremely low birth weight infants. *Pediatrics*. 2006;117(1):84–92.
21. World Health Organization. *Global report on the epidemiology and burden of sepsis*. Geneva: WHO; 2020.
22. Wadile RG, Bhate VM. Clinical spectrum and risk factors of neonatal candidemia. *Indian J Pathol Microbiol*. 2015;58(4):472–474.
23. Sardana V, Pandey A, Madan M, Goel SP, Asthana AK. Neonatal candidemia: a changing trend. *Indian J Pathol Microbiol*. 2012;55(1):132–133.
24. Basu S, Kumar R, Tilak R, Kumar A. Candida bloodstream infections in neonates. *Indian Pediatr*. 2017;54(7):556–559.
25. Fu J, Ding Y, Ba W, Wang L, Xu S, Qin P, et al. Epidemiology of neonatal candidemia in western China. *BMC Infect Dis*. 2017; 17:329.
26. Nakasone I, Kinjo T, Yamane N, Kisanuki K, Shiohira CM. Evaluation of the VITEK-2 Compact system. *Diagn Microbiol Infect Dis*. 2007;58(2):191–198.
27. Jahan T, Farhana A, Nargis S. Persisting non-albicans candidemia in low-birth-weight neonates. *Int J Res Med Sci*. 2023; 11:1514–1520.
28. Wadile RG, Vasave RA, Kokani VR. Risk factors and biofilm formation among non-albicans Candida in neonatal candidemia. *IP Int J Med Microbiol Trop Dis*. 2025;11(3):270–273.
29. Singh A, Mukhopadhyay S. Changing trends of candida sepsis in NICU. *Arch Dis Child*. 2022;107(Suppl 2): A149–A150.
30. Deorukhkar SC, Saini S, Mathew S. Non-albicans Candida infection: an emerging threat. *Interdiscip Perspect Infect Dis*. 2014; 2014:615958.
31. Bliss JM, Basavegowda KP, Watson WJ, Sheikh AU, Ryan RM. Transmission of Candida albicans in very low birth weight infants. *Pediatr Infect Dis J*. 2008;27(3):231–235.
32. Ali GY, Algohary EHS, Rashed KA, Almoghanum M, Khalifa AAR. Candida colonization in preterm newborns. *J Matern Fetal Neonatal Med*. 2012;25(6):789–795.
33. Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. *Virulence*. 2013;4(2):119–128.
34. Bhatt M, Sarangi G, Paty BP, Mohapatra D, Chayani N, Mahapatra A, et al. Biofilm as a virulence marker in Candida species. *Indian J Med Microbiol*. 2015;33(1 Suppl):112–114.
35. Nazir A. Non-albicans Candida in neonatal septicemia. *Int J Biomed Res*. 2016;7(2):47–50.
36. Bansal R, Oberoi L, Singh K, Devi P. Candida species distribution and biofilm formation in NICU patients. *Int J Curr Microbiol App Sci*. 2016;5(12):628–634.
37. Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the VITEK 2 yeast susceptibility test. *J Clin Microbiol*. 2007;45(3):796–802.
38. Hernández-Pabón JC, Tabares B, Gil O, et al. Non-albicans Candida causing invasive candidiasis. *J Fungi*. 2024; 10:326.
39. Chávez JF, Ortiz B, López R, et al. Virulence factors of Candida species causing candidemia. *J Fungi*. 2025; 11:470.
40. Zhang D, Xie D, Yuan H, He N, Dong W, Lei X. Managing invasive Candida infections in hospitalized newborns. *Front Pediatr*. 2025; 13:1613832.
41. Sachin CD, Ruchi K, Santosh S. In vitro evaluation of virulence enzymes of Candida species. *Int J Med Biomed Res*. 2012;1(2):153–157.
42. Sieben RG, Paternina-de la Ossa R, Waack A, et al. Risk factors and mortality of candidemia in children. *Rev Argent Microbiol*. 2024;56(3):281–286.
43. Sandhu R, Dahiya S, Sayal P, Budhani D. Non-albicans Candida and attributable mortality. *J Health Res Rev*. 2017;4(2):78–83.