RESEARCH PAPER

Molecular Detection of MDR1 and ERG11 Genes and Antifungal Susceptibility Profiles of Candida Species Isolated from Clinical Samples

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Received: 24th April, 2026; Revised: 20th May 2026; Accepted: 30th June, 2026; Available Online: 10th July, 2026

ABSTRACT
Candida infections remain a significant cause of illness among hospitalized patients, and there has been a notable shift toward non-albicans Candida (NAC) species along with rising antifungal resistance. In this study, we looked at the species distribution, antifungal susceptibility patterns, key virulence factors, and the presence of MDR1 and ERG11 genes in clinical Candida isolates. We collected 150 Candida isolates from a range of clinical specimens and identified them phenotypically using CHROMagar and standard biochemical methods. Susceptibility to fluconazole, voriconazole, caspofungin, micafungin, and flucytosine was tested using the VITEK 2 system. Biofilm formation was checked using tube and tissue-culture plate methods and siderophore production was assessed on chrome azurol S agar. For isolates resistant to fluconazole, we used PCR to check for the MDR1 (835 bp) and ERG11 (1587 bp) genes.

Candida tropicalis emerged as the most common species (67/150; 44.7%), followed by C. albicans (51/150; 34.0%). We found fluconazole resistance in 61 isolates overall, including 33 C. albicans and 14 C. tropicalis. Biofilm formation was seen in 48 isolates (32.0%), with C. albicans accounting for the largest share. None of the isolates showed siderophore production. Among the fluconazole-resistant isolates, MDR1 alone was found in 10, ERG11 alone in 17, and both genes together in 4. Our findings showed the predominance of NAC species particularly C. tropicalis along with a considerable burden of azole resistance among the Candida isolates studied. The presence of MDR1 and ERG11 in some resistant isolates suggests these genes contribute to azole resistance, but their absence in many other resistant isolates points to additional, likely multifactorial, mechanisms of resistance at play.

Keywords: Candida; non-albicans Candida; antifungal resistance; fluconazole; MDR1; ERG11; biofilm; azole resistance

How to cite this article: Surender, Bala R, Aman S, Kaur N, Kumar D, Molecular Detection of MDR1 and ERG11 Genes and Antifungal Susceptibility Profiles of Candida Species Isolated from Clinical Samples. Int J Drug Deliv Technol. 2026;16(7): 1132-1142; DOI: 10.25258/ijddt.16.7.120
Source of support: Nil.
Conflict of interest: None

1. INTRODUCTION

Candida species are among the most common opportunistic fungal pathogens in humans, responsible for a wide spectrum of infections ranging from superficial mucosal lesions to life-threatening systemic diseases [1]. *Candida albicans* was considered the principal etiological agent however, over the past few decades, non-*albicans Candida* (NAC) species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei* have emerged as major contributors to candidiasis, particularly in immunocompromised and hospitalized patients [2]. This epidemiological shift, first noted in the late 20th century, is largely driven by extensive use of broad-spectrum antibiotics and antifungals, immunosuppressive therapies, and invasive medical procedures. These interventions disrupt normal flora and create selective pressure that favors NAC species, many of which possess intrinsic or acquired antifungal resistance [3]. Infections are especially prevalent in intensive care units, oncology wards, and among neonates, transplant recipients, and individuals with HIV/AIDS. Geographic variation is evident, with *C. tropicalis* predominating in Asian regions and *C. glabrata* being more frequent in North America and Europe [4]. The clinical importance of NAC species stems from their unique virulence traits and reduced antifungal susceptibility. Unlike *C. albicans*, which relies primarily on hyphal formation for invasion, NAC species exhibit diverse pathogenic mechanisms. *C. glabrata* and *C. tropicalis* form robust biofilms, secrete hydrolytic enzymes such as proteases and phospholipases, and modify their cell wall composition to evade immune recognition. These adaptations facilitate persistent colonization, enhance survival under antifungal stress, and contribute to therapeutic failure [5]. Antifungal resistance

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among NAC species is a growing global concern. *C. glabrata* often demonstrates reduced susceptibility to azoles through efflux pump overexpression and mutations in ergosterol biosynthesis genes, while *C. krusei* is inherently resistant to fluconazole. The emergence of multidrug-resistant strains in hospital settings further complicates management, limiting therapeutic options and increasing mortality risk [6]. Consequently, species-specific antifungal susceptibility testing has become essential for guiding appropriate therapy. Accurate and timely diagnosis remains a major challenge. Conventional culture-based methods and biochemical assays frequently misidentify NAC species or overlook mixed infections, leading to inappropriate treatment. In contrast, advanced molecular tools such as MALDI-TOF mass spectrometry and PCR-based assays enable rapid and precise identification [7].

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This experimental study was conducted in the Department of Microbiology at Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Haryana, India. The study was designed to evaluate the phenotypic and genotypic characteristics of *Candida* species isolated from various clinical specimens.

2.2 Study Population and Sample Size

The study population consist of *Candida* isolates recovered from different clinical samples of patients attending MMIMSR. The sample size was determined using the formula ($N= Z^2PQ/E^2$, where $Z = 1.96$ at a 95% confidence level (1.96), $P = 9\%$, $Q = 1-P$ and $E = 4.58\%$ as allowable margin of error. The calculated minimum number of isolates was 150.

2.3 Inclusion and Exclusion Criteria

Only those isolates that were phenotypically confirmed as *Candida* species were included in the study. Patients who were already receiving antifungal therapy at the time of sample collection were excluded to avoid bias in antifungal susceptibility results.

2.4 Ethical Approval

Ethical approval for the study was obtained from the Institutional Ethics Committee of MMIMSR, Mullana, Haryana (IEC No. IEC/2782 dated 08/12/2023). All procedures were carried out in accordance with institutional ethical standards.

2.5 Specimen Collection and Processing

Clinical samples including blood, urine, sputum, pus, vaginal swabs, bronchoalveolar lavage, cerebrospinal fluid and other body fluids were collected under aseptic conditions. Initial processing involved direct microscopic examination using potassium hydroxide mount and Gram staining. Samples were cultured on Sabouraud's Dextrose Agar and CHROMagar *Candida* media and incubated at 37°C for 24–48 hours to facilitate growth and preliminary identification of yeast colonies.

2.6 Identification of *Candida* Species

Candida isolates were identified based on colony morphology on CHROMagar, germ tube test, and carbohydrate assimilation and fermentation tests. The characteristic colony colors observed on CHROMagar aided in presumptive identification, which was further confirmed using biochemical methods to achieve species-level identification [8].

2.7 Antifungal Susceptibility Testing

Antifungal susceptibility testing of all isolates was performed using the VITEK 2 automated system. The antifungal agents tested included fluconazole, voriconazole, caspofungin, micafungin, and flucytosine. The results were interpreted according to CLSI guidelines to determine resistance patterns among different *Candida* species [9].

2.8 Detection of Virulence Factors

Biofilm formation was assessed using both the test tube method and the tissue culture plate method. Based on the intensity of biofilm formation, isolates were categorized as non, weak, moderate, or strong biofilm producers. In addition, siderophore production was evaluated using the modified Chrome Azurol S agar assay, where a change in color indicated positive siderophore production [10].

2.9 Molecular Detection of Resistance Genes

2.9.1 MDR1 Gene Amplification

The *MDR1* gene, encoding a drug efflux pump associated with azole resistance, were amplified by polymerase chain reaction (PCR) using the primers: Forward (5′-AGGTGAACCCAATTCAAGTC-3′) and Reverse (5′-ACAACTGGTTCCATAACGGT-3′). PCR will be carried out in 25 µL reaction mixtures containing template DNA, primers (1 µL each), and other reagents. The amplification protocol included initial denaturation at 95°C for 30 s, followed by 30 cycles of annealing at 57°C for 1 min and extension at 72°C for 1 min. Amplified products (MDR1 gene 835 bp) were analysed by agarose gel electrophoresis using 0.8% agarose and visualized under UV light [11].

2.9.2 ERG11 Gene Amplification

The complete coding region of the *ERG11* gene were amplified from resistant and susceptible isolates using the primers: Forward (5′-CAAGAAGATCATAACTCAAT-3′) and Reverse (3′-AGAACACTGAATCGAAAG-5′). PCR reactions were performed in 20 µL mixtures containing Maxime PCR PreMix Kit (i-Taq™ polymerase, dNTPs, and buffer), primers, and 2.5 µL genomic DNA. The cycling conditions included initial denaturation at 94°C for 4 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 70°C for 1 min, with a final extension at 72°C for 10 min. The amplified products (ERG11 Gene 1587 bp) were run on 0.8% agarose gels, visualized under UV illumination, and photographed [12].

3. RESULTS

3.1 Department-wise Distribution

A total of 150 *Candida* isolates were obtained from patients admitted across various hospital departments. The highest number of isolates was reported from the Medicine department (34 isolates), followed by the Neonatal Intensive Care Unit (32 isolates) and Gynaecology department (26 isolates). Other departments contributing to the isolates included Chest and TB (18 isolates), Pediatric Critical Care Medicine (16 isolates), Trauma ICU (12 isolates), and smaller contributions from ENT and Pediatrics with 6 isolates each **(Table 1)**.

Table 1: Department wise distribution of *Candida* isolates.

Department	No. of Isolates
Chest & TB	18
ENT	6
Med	34
Gynae	26
NICU	32
Paeds	6
PCCM	16
TICU	12
Total	**150**

3.2 Specimen-wise Distribution

Among the collected clinical samples, blood was the most common source of *Candida* isolates, accounting for 62 isolates. This was followed by vaginal swabs (26 isolates), urine samples (16 isolates), and both pus and sputum samples (12 isolates each). Bronchoalveolar lavage contributed 10 isolates, while cerebrospinal fluid accounted for 5 isolates. Ascitic fluid, tissue biopsy, and PCCM samples contributed minimally, with one isolate each **(Table 2)**.

Table 2: Distribution of Specimens collected from Patients with *Candida* infection

Sample Type	No. of Sample
Ascitic Fluid	1
BAL	10
Blood	62
CSF	5
ENT Swab	4
PCCM	1
Pus	12
Sputum	12
Tissue Biopsy	1
Urine	16
Vaginal Swab	26
Total	**150**

3.3 Gender Distribution

The study population showed a predominance of female patients, with 103 cases (68.7%), compared to 47 cases (31.3%) in males. This indicates a higher burden of *Candida* infections among female patients in the studied cohort.

3.4 Species Distribution

The distribution of *Candida* species revealed a predominance of non-albicans *Candida*. *Candida tropicalis* was the most frequently isolated species, accounting for 67 cases (44.7%), followed by *Candida albicans* with 51 cases (34%). Other species included *Candida pelliculosa* and *Candida parapsilosis* with 12 cases each, *Candida* auris with 7 cases, and rare isolates such as *Candida ceffferi* and *Candida krusei* with one case each **(Table 3 & Fig. 1)**.

Fig. 1 Picture showing the speciation of *Candida* species using chrome agar.

Table 3: Distribution of *Candida* species obtained from patients	
Isolate	**No. of isolates**
Candida albicans	51
Candida auris	7
Candida ceffferi	1
Candida krusei	1
Candida pelliculosa	12
Candida prapsilosis	12
Candida tropicallis	67
Total	**150**

3.5 Antifungal Resistance Profile

Antifungal susceptibility testing demonstrated a high level of resistance to azole antifungals. *Candida* albicans showed resistance to fluconazole in 33 isolates and to voriconazole in 36 isolates. *Candida* tropicalis exhibited fluconazole resistance in 14 isolates, while *Candida* auris showed resistance in 5 isolates. In contrast, resistance to echinocandins such as micafungin and caspofungin was minimal, indicating their continued effectiveness against most isolates **(Table 4)**.

Table 4: Antifungal Resistance Profile of *Candida* species obtained from Patients.					
Isolates	**Fluconazole**	**Voriconazole**	**Caspofungin**	**Micafungin**	**Flucytosine**
Candida albicans (n-51)	33	36	36	0	11
Candida auris (n-7)	5	4	2	3	11
Candida ceffferi (n-1)	0	0	0	0	0
Candida krusei (n-1)	0	0	0	0	0
Candida pelliculosa (n-12)	4	4	4	2	6
Candida prapsilosis (n-12)	5	0	2	0	4
Candida tropicallis (n-67)	14	0	0	0	14

3.6 Biofilm Production

Out of the 150 isolates, 102 (68%) were non-biofilm producers, whereas 48 isolates demonstrated biofilm-forming ability. Among these, 35 isolates were strong biofilm producers, while 9 and 4 isolates were categorized as moderate and weak producers, respectively. Species-wise analysis revealed that *Candida albicans* contributed the highest number of biofilm producers (29 isolates), followed by *Candida tropicalis* (18 isolates), while other species showed minimal or no biofilm formation **(Table 5 & Fig. 2)**.

Table 5: Distribution of Biofilm Producing & Non-Biofilm Producing *Candida* species obtained from patients

Isolates	Total No. of isolates	No. of Biofilm Producers
Candida albican	51	29
Candida auris	7	0
Candida ceffferi	1	0
Candida krusei	1	0
Candida pelliculosa	12	0
Candida prapsilosis	12	1
Candida tropicallis	67	18
Total	**150**	**48**

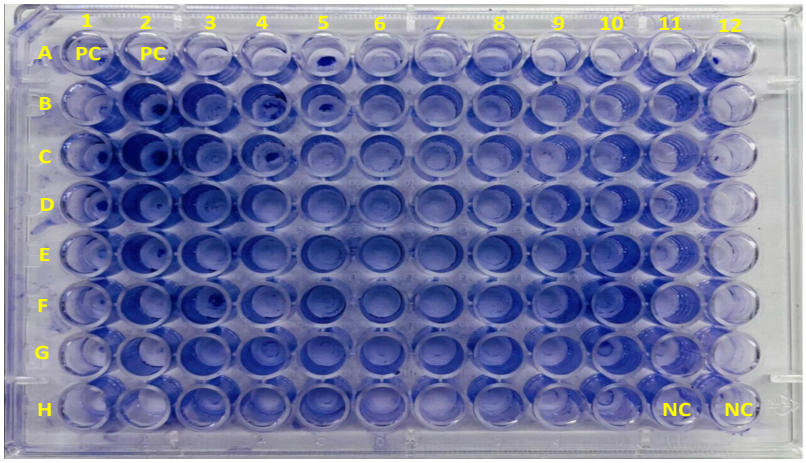

Fig. 2 Picture showing the biofilm formation by *Candida* species using 96 well tissue culture plate.

Isolates were tested for biofilm production in duplicates in vertical row layout. Well No. A1 & 2 were positive control (Biofilm Producing *Acinetobacter baumannii* an in-house control whereas well no. H 11 & 12 were negative control without any isolates)

3.7 Siderophore Production

siderophore production as a virulence factor contributing to iron acquisition and pathogenicity in *Candida* species Siderophore production was evaluated among recruited *Candida* isolates using the CAS agar assay. Visible color change from blue to pink in the medium was observed in case of positive siderophore production. No *Candida* species were identified as siderophores producer **(Fig. 3)**.

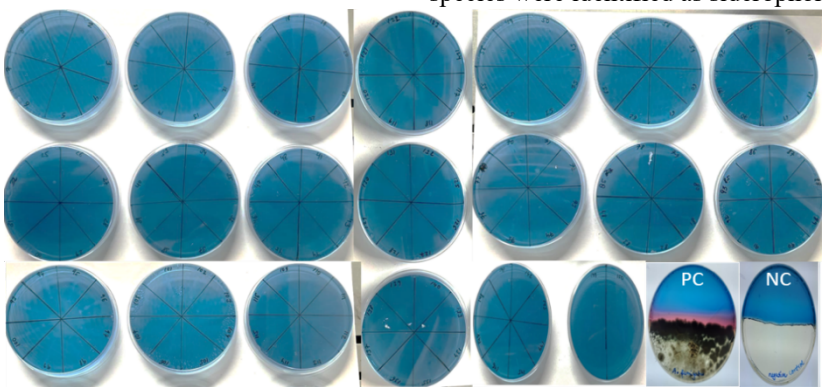

Fig. 3 Detection of siderophores production by *Candida* species using CAS-agar assay.

PC- Positive control (*Aspergillus fumigatus* an in-house control, NC- Negative control without any culture.

3.8 Molecular Detection of Resistance Genes

Molecular analysis of fluconazole-resistant isolates revealed the presence of MDR-1 and ERG-11 genes.

Among *Candida albicans* isolates, MDR-1 was detected in 6 and ERG-11 in 10 isolates, while co-expression of both gene was observed in 3 isolates. In *Candida tropicalis*, MDR-1 was detected in 4 isolates, ERG-11 in 7 isolates while co-expression of both gene was observed in one isolate. Furthermore, no MDR-1 & ERG-11 genes were observed in other *Candida* species. These findings confirm the genetic basis of antifungal resistance among selected *Candida* isolates **(Table 6 & Fig. 4)**.

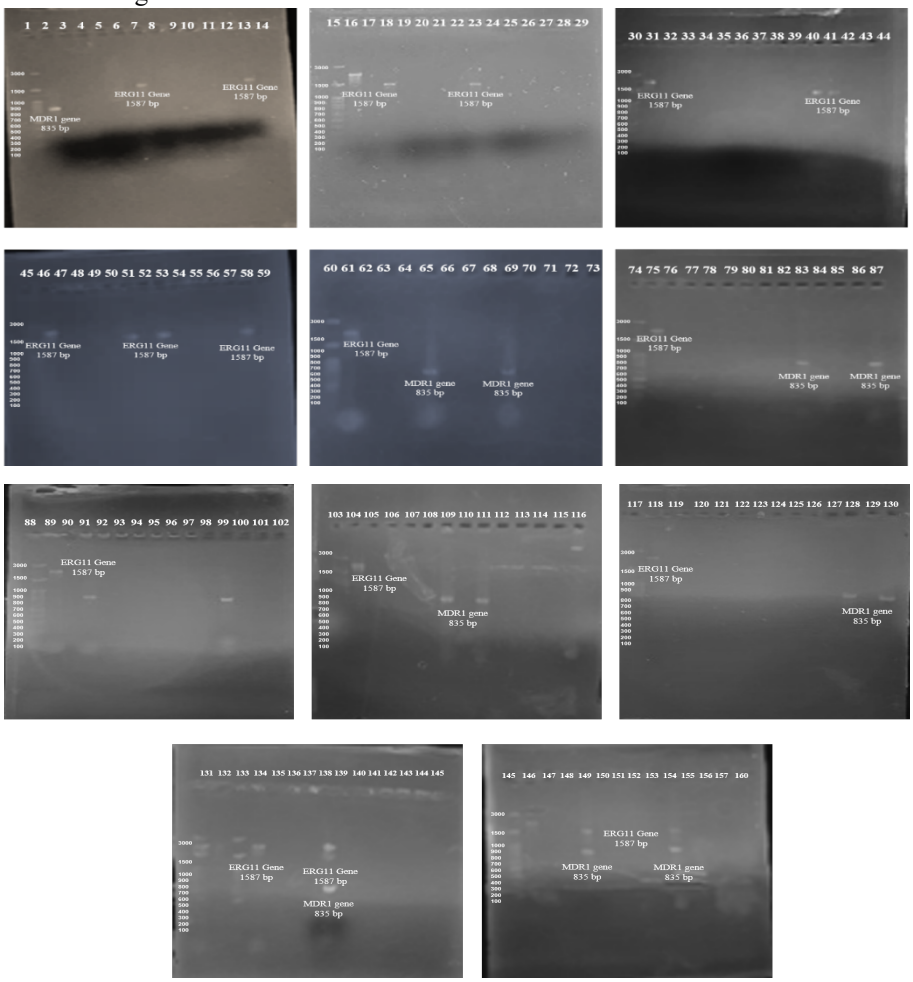

Fig. 4 PCR amplification of ERG11 and MDR1 genes among Candida isolates.

Agarose gel electrophoresis showing PCR amplification products of the ERG11 gene (1587 bp) and MDR1 gene (835 bp) in *Candida* isolates. Bands corresponding to the expected amplicon sizes of 1587 bp (ERG11) and 835 bp (MDR1) indicate positive amplification of the respective genes. A molecular DNA ladder of 100 bp was used for estimation of amplicon size.

Table 6: Antifungal Resistance Profile of *Candida* species obtained from Pateints

Isolates	No. of Fluconazole Resistant Isolates	No. of Isolates Carrying Only MDR-1 gene	No. of Isolates Carrying Only ERG-11 gene	No. of Isolates having Co-Expression of MDR-1 & ERG-11 Gene
Candida albicans	33	6	10	3
Candida auris	5	0	0	0
Candida ceffferi	0	0	0	0
Candida krusei	0	0	0	0
Candida pelliculosa	4	0	0	0
Candida prapsilosis	5	0	0	0
Candida tropicallis	14	4	7	1
Total	**61**	**10**	**17**	**4**

DISCUSSION

This study characterised 150 clinical *Candida* for the phenotypic, susceptibility, virulence and molecular levels

obtained from patients reported at tertiary-care centres in Haryana. Three broad findings emerged: a numerical predominance of non-albicans *Candida* (NAC) species led by *Candida tropicalis*; a high burden of azole resistance concentrated in *C. albicans* and *C. tropicalis* alongside comparatively preserved echinocandin activity; and a molecular resistance signature involving MDR1 efflux-pump and ERG11 alterations in only a subset of phenotypically resistant isolates. Each of these findings is discussed below against comparable Indian and international literature.

The predominance of *C. tropicalis* (44.7%) over *C. albicans* (34%) in the present cohort places these centres at the more advanced end of a transition that has been documented across India over the past two decades. A tertiary-care series from South India similarly reported that non-albicans species collectively outnumbered *C. albicans*, although *C. albicans* remained the single most common individual isolate (46.4%) with *C. tropicalis* a distant second (25.8%)[13]. A large retrospective analysis from Southern India encompassing 751 samples found an even more pronounced NAC predominance (73.64%), with *C. tropicalis* again the leading non-albicans species (42.88%)[14], while a Northern Indian tertiary-care study reported *C. albicans* as still the most common overall isolate, with *C. tropicalis* the leading NAC species and the neonatal intensive care unit contributing disproportionately to case numbers [15], a pattern that mirrors the present study's own department-wise distribution. A separate Indian series characterising virulence factors of non-*C. albicans Candida* species similarly found NAC species (59.9%) outnumbering *C. albicans*, with *C. tropicalis* contributing the largest share among NAC isolates [16]. Taken together, these studies confirm that *C. tropicalis* has become the dominant non-albicans pathogen across multiple Indian regions, though the point at which it overtakes *C. albicans* as the single leading isolate as occurred in this cohort varies by centre, patient case-mix and the proportion of ICU, oncology and neonatal referrals served.

The detection of *Candida* auris in seven isolates (4.7%), five of which were fluconazole-resistant, is consistent with its recognised emergence as a multidrug-resistant nosocomial pathogen since its first description from a Delhi hospital; a multinational collaborative surveillance effort that included Indian isolates reported fluconazole resistance in 93% of *C. auris* strains, with lower but non-negligible resistance to amphotericin B and, less frequently, echinocandins [17]. A recent Indian ICU-based study similarly described *C. auris* as an emerging concern in critical-care settings, reinforcing the plausibility of its detection among the ICU- and NICU-heavy specimen mix seen here [18]. Blood was the leading specimen type (41.3%), and the Medicine, NICU and Gynaecology departments together accounted for more than half of all isolates. This distribution is consistent with the growing recognition of candidemia as a major nosocomial bloodstream infection in Indian hospitals. A central Indian tertiary-care study of blood-culture isolates over one year found *C. tropicalis* to be the leading cause of candidemia (32.2%), followed by *C. albicans* and *C. parapsilosis* [19], while a North Indian paediatric-weighted cohort from New Delhi similarly identified *C. tropicalis* as the most common bloodstream isolate and reported a male predominance (60.9%) driven largely by neonatal admissions [20]. A three-year study from Western India found that non-albicans species accounted for 89% of *Candida*emia isolates, again led by *C. tropicalis* [21], and an earlier North Indian series reported a comparably high fluconazole resistance rate of 77.8% among candidemia isolates, closely paralleling the resistance burden observed in the present study [22]. A trauma-centre-based Indian study extended this picture to a younger, comorbidity-light population, underscoring that candidemia risk in Indian hospitals is not confined to the classically described elderly or immunosuppressed groups [23]. The heavy contribution of the NICU in the present series (32 isolates, 21.3% of the cohort) is also in keeping with a broader literature identifying prematurity, central catheterisation, prolonged broad-spectrum antibiotic exposure, parenteral nutrition and mechanical ventilation as the principal drivers of invasive candidiasis in neonatal populations [24]. The marked female predominance observed here (68.7%) contrasts with several blood-culture-focused Indian candidemia cohorts, which have reported a male predominance driven by higher rates of male neonatal and ICU admissions [20]. This apparent discrepancy is best explained by specimen composition rather than a difference in underlying susceptibility: vaginal swabs constituted the second largest specimen category in this study (17.3%), and vulvovaginal candidiasis is a condition of reproductive-age women, driven by oestrogen-related glycogen deposition that promotes *Candida* adherence and germination on the vaginal epithelium. A twenty-year Italian surveillance study of vulvovaginal candidiasis found the highest prevalence in women aged 18–30 years [25], and an Ethiopian study of symptomatic vulvovaginal candidiasis similarly reported *C. albicans* as the leading isolate among a predominantly reproductive-age female population [26]. The present study's overall female predominance therefore most plausibly reflects the substantial genital-specimen contribution to the sample rather than a generalised sex-based susceptibility difference across all clinical syndromes captured. Azole resistance was substantial in this cohort, particularly among *C. albicans* isolates (64.7% fluconazole-resistant, 70.6% voriconazole-resistant). This exceeds the combined acquired-plus-intrinsic fluconazole resistance rate of roughly 40% reported in a South Indian cross-sectional study [13], though it is broadly concordant with the 77.8% fluconazole resistance reported among candidemia isolates in an earlier North Indian series [22]. Notably, several other Indian series have described *C. tropicalis*, rather than *C. albicans*, as carrying the heavier azole-resistance burden for example, a central Indian bloodstream-isolate study found *C. tropicalis* exhibiting the highest resistance among all species tested, particularly to posaconazole [19].

In the present cohort, however, *C. albicans* isolates showed proportionally greater resistance than *C. tropicalis* (20.9% fluconazole-resistant). This divergence from the more commonly reported NAC-dominant resistance pattern is worth highlighting explicitly in the manuscript, since it runs somewhat against the prevailing narrative that non-albicans species are disproportionately responsible for treatment-limiting azole resistance; it may reflect the specific antifungal exposure history of the referral population, strain-level heterogeneity, or the categorical (VITEK 2) rather than MIC-based nature of the susceptibility data, and would benefit from confirmation with broth microdilution testing in future work. The overall preservation of echinocandin activity described in the Results is consistent with the global experience captured by large multi-year surveillance programmes: a six-year international surveillance study of more than 5,000 invasive *Candida* isolates found that all three echinocandins inhibited over 99% of isolates, including *C. albicans* and *C. tropicalis*, with resistance essentially confined to occasional *C. glabrata* strains carrying fks mutations [27], a pattern echoed in a subsequent review of echinocandin resistance epidemiology [28]. This concordance supports the continued use of echinocandins as a reasonable first-line empirical choice for suspected invasive candidiasis at these centres, particularly given the high azole resistance documented above.

One internal inconsistency in Table 6 warrants attention before this manuscript is finalised: the table records caspofungin resistance in 36 of 51 (70.6%) *C. albicans* isolates, which is difficult to reconcile both with the manuscript's own narrative statement that echinocandin resistance was minimal, and with the near-universal global experience of echinocandin activity against *C. albicans* summarised above [27,28]. Given the magnitude of the discrepancy, a transcription or column-alignment error in the table seems more likely than a genuine, previously unreported caspofungin-resistant *C. albicans* cluster — but if the raw VITEK 2 data do confirm this figure, it would itself be a notable and separately reportable finding requiring confirmatory MIC testing and FKS gene sequencing before publication. Thirty-two percent of isolates produced biofilm in this study, with *C. albicans* contributing the largest absolute number of biofilm-positive isolates (29/51, 56.9%) compared with *C. tropicalis* (18/67, 26.9%). This finding sits in contrast to another Indian series, which reported a higher proportional biofilm-forming rate in *C. tropicalis* (78.1%) than in *C. albicans* (67%) [16], and to a virulence-factor study of *C. tropicalis* that linked biofilm and phospholipase production to greater fluconazole resistance within that species [29]. The comparatively stronger biofilm phenotype of *C. albicans* in the present cohort is, however, consistent with the classical understanding of the genus: *C. albicans* possesses a more elaborate hyphal and pseudohyphal morphogenetic programme that underpins particularly robust biofilm architecture, a trait less uniformly developed across NAC species. The divergence from other Indian NAC-dominant cohorts most likely reflects differences in biofilm assay methodology (combined tube and tissue-culture-plate methods here versus quantitative XTT or crystal-violet approaches elsewhere), inoculum standardisation and incubation conditions, all recognised sources of inter-study variability in biofilm phenotyping, rather than a true biological discrepancy. The detection of siderophore activity by the CAS-agar assay in this study is presented as evidence of iron-acquisition-related virulence. An important nuance from the mycological literature should be incorporated into this discussion: unlike many bacterial pathogens, *Candida* species including *C. albicans* are generally not considered de novo siderophore producers. Established work has shown that *C. albicans* instead relies predominantly on a reductive, ferric-reductase-dependent high-affinity iron-uptake system together with heme and haemoglobin acquisition pathways, and separately exploits siderophores secreted by other microorganisms (xenosiderophores) via a dedicated transporter rather than synthesising its own siderophores [30–32]. A positive colour change on CAS agar in a *Candida* isolate should therefore be interpreted with some caution: it may reflect iron-chelating activity of other secreted metabolites or uptake-related surface activity rather than de novo siderophore biosynthesis in the sense typically implied for bacteria. Corroborating the CAS-assay result with a confirmatory chemical assay, or with expression analysis of the reductive iron-uptake and xenosiderophore-transport genes, would considerably strengthen this component of the virulence characterisation and should be considered for any follow-up work arising from this dataset.

Among fluconazole-resistant *C. albicans* isolates, ERG11 was detected more frequently than MDR1 alone (10 versus 6 isolates), with co-expression of both genes in only three isolates; a broadly similar pattern was seen in *C. tropicalis*. These two mechanisms are the best-characterised routes to azole resistance in *Candida*: MDR1 encodes a major-facilitator-superfamily efflux pump whose overexpression, driven by the transcription factor MRR1, is sufficient on its own to confer clinically relevant fluconazole resistance [33–35], while ERG11 mutations or overexpression reduce the affinity or increase the abundance of the azole target enzyme, sterol 14α-demethylase; ERG11 mutation has been identified as the principal resistance mechanism in oropharyngeal *C. albicans* isolates from head-and-neck cancer patients [36]. In *C. tropicalis*, combined MDR1 overexpression and ERG11 mutation has been repeatedly implicated as the dominant azole-resistance mechanism in Indian cohorts: a case report described a Y132C ERG11 substitution together with MDR1 overexpression driving pan-azole resistance in a leukaemia patient [37], a study of clinical *C. tropicalis* isolates found ERG11 missense mutations strongly correlated with elevated MDR1 expression in high-level and pan-azole-resistant strains [38], and a North Indian study reported coordinated overexpression of MDR1, ERG11 and CDR1 in resistant *C. tropicalis* isolates [39], a finding corroborated by subsequent expression-profiling work in the same species [40]. The present study's pattern each gene independently

more prevalent than their co-expression, and a majority of phenotypically fluconazole-resistant isolates in both species testing negative for both gene is consistent with this literature's central conclusion that azole resistance in *Candida*, and particularly in NAC species, is typically multifactorial. It follows that a two-gene panel restricted to MDR1 and ERG11 will necessarily under-explain the full resistant phenotype, and that additional mechanisms not assessed here CDR1/CDR2 efflux-pump overexpression, ERG3 mutation, biofilm-associated drug tolerance, and chromosomal aneuploidy likely account for resistance in the gene-negative isolates.

CONCLUSION

This study well-established Indian trend toward non-albicans *Candida* predominance, led by *C. tropicalis*, alongside a substantial and clinically important burden of azole resistance that is only partly explained by MDR1 and ERG11 alterations. Preserved echinocandin activity subject to verification of the caspofungin discrepancy noted above continues to support echinocandins as a reliable empirical option pending susceptibility results. Continued species-level surveillance, ideally supplemented by MALDI-TOF or sequencing-based confirmation and a broader molecular resistance panel, will be important for tracking whether the resistance and virulence patterns identified here represent a stable local epidemiology or a continuing shift.

REFERENCES

[1] Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, Et Al. Candida Albicans—The Virulence Factors And Clinical Manifestations Of Infection. Journal Of Fungi 2021;7:79. Https://Doi.Org/10.3390/Jof7020079.

[2] Gómez-Gaviria M, Ramírez-Sotelo U, Mora-Montes Hm. Non-Albicans Candida Species: Immune Response, Evasion Mechanisms, And New Plant-Derived Alternative Therapies. Journal Of Fungi 2022;9:11. Https://Doi.Org/10.3390/Jof9010011.

[3] Parslow By, Thornton Cr. Continuing Shifts In Epidemiology And Antifungal Susceptibility Highlight The Need For Improved Disease Management Of Invasive Candidiasis. Microorganisms 2022;10:1208. Https://Doi.Org/10.3390/Microorganisms10061208.

[4] Sharma M, Chakrabarti A. Candidiasis And Other Emerging Yeasts. Curr Fungal Infect Rep 2023;17:15–24. Https://Doi.Org/10.1007/S12281-023-00455-3.

[5] Pawar Sa, Karande Gs, Patil Sr. From Identification To Resistance: Contemporary Insights Into Candida Species Causing Vaginitis. Cureus 2026. Https://Doi.Org/10.7759/Cureus.108327.

[6] Whaley Sg, Berkow El, Rybak Jm, Nishimoto At, Barker Ks, Rogers Pd. Azole Antifungal Resistance In Candida Albicans And Emerging Non-Albicans Candida Species. Front Microbiol 2017;7. Https://Doi.Org/10.3389/Fmicb.2016.02173.

[7] 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;30:42. Https://Doi.Org/10.1186/S12929-023-00926-2.

[8] Murray Pr. The Clinician And The Microbiology Laboratory. Mandell, Douglas, And Bennett's Principles And Practice Of Infectious Diseases, Elsevier; 2015, P. 191–223. Https://Doi.Org/10.1016/B978-1-4557-4801-3.00016-3.

[9] Siopi M, Pachoulis I, Leventaki S, Spruijtenburg B, Meis Jf, Pournaras S, Et Al. Evaluation Of The Vitek 2 System For Antifungal Susceptibility Testing Of Candida Auris Using A Representative International Panel Of Clinical Isolates: Overestimation Of Amphotericin B Resistance And Underestimation Of Fluconazole Resistance. J Clin Microbiol 2024;62. Https://Doi.Org/10.1128/Jcm.01528-23.

[10] Ranđelović M, Dimitrijević M, Mijatović S, Ignjatović A, Arsić-Arsenijević V, Stojanović-Radić Z, Et Al. Antifungal Susceptibility And Biofilm Production Of Candida Species– Causative Agents Of Female Genital Tract Infections. Brazilian Journal Of Microbiology 2024;55:3863–72. Https://Doi.Org/10.1007/S42770-024-01529-1.

[11] Dhasarathan P, Alsalhi Ms, Devanesan S, Subbiah J, Ranjitsingh Aja, Binsalah M, Et Al. Drug Resistance In Candida Albicans Isolates And Related Changes In The Structural Domain Of Mdr1 Protein. J Infect Public Health 2021;14:1848–53. Https://Doi.Org/10.1016/J.Jiph.2021.11.002.

[12] Osman Mohamed A, Suliman Mohamed M, Abdelrahman Hussain M, Fatahalrahman Ahmed I. Detection Of Antifungal Drug-Resistant And Erg11 Gene Mutations Among Clinical Isolates Of Candida Species Isolated From Khartoum, Sudan. F1000res 2021;9:1050. Https://Doi.Org/10.12688/F1000research.24854.2.

[13] M A, Jd J. Species Distribution And Antifungal Susceptibility Patterns Of Candida Isolates: A Cross-Sectional Study From A Tertiary Care Hospital In South India. Cureus 2025. Https://Doi.Org/10.7759/Cureus.79666.

[14] S. U, Sumana Mn. Retrospective Analysis On Distribution And Antifungal Susceptibility Profile Of Candida In Clinical Samples: A Study From Southern India. Front Public Health 2023;11. Https://Doi.Org/10.3389/Fpubh.2023.1160841.

[15] Kaur P, Rawat T, Sharma S, Kaur P. Speciation Of Candida Species Isolated In Clinical Samples In A Tertiary Health Care Centre In Northern India. Ip International Journal Of Medical Microbiology And Tropical Diseases 2021;7:262–8. Https://Doi.Org/10.18231/J.Ijmmtd.2021.054.

[16] Prasad Singh D, Kumar Verma R, Sarswat S, Saraswat S. Non-Candida Albicans Candida Species: Virulence Factors And Species Identification In India. Curr Med Mycol 2021. Https://Doi.Org/10.18502/Cmm.7.2.7032.

[17] Chowdhary A, Sharma C, Meis Jf. Candida Auris: A Rapidly Emerging Cause Of Hospital-Acquired Multidrug-Resistant Fungal Infections Globally. Plos Pathog 2017;13:E1006290. Https://Doi.Org/10.1371/Journal.Ppat.1006290.

[18] Singh P, Pandey A. Emergence Of Multidrug-Resistant And Opportunistic Fungal Infections Due To Candida Auris In Intensive Care Units. Curr Med Mycol 2024;10. Https://Doi.Org/10.22034/Cmm.2024.345249.1558.

[19] Bhadade Aa, Singh G, Karuna T, Panthi M, Beniwal A, Maurya Ak, Et Al. Epidemiology And Antifungal Susceptibility Of Candida Bloodstream Isolates From A Tertiary Care Center In Central India. Cureus 2025. Https://Doi.Org/10.7759/Cureus.97120.

[20] Gautam G, Rawat D, Kaur R, Nathani M. Candidemia: Changing Dynamics From A Tertiary Care Hospital In North India. Curr Med Mycol 2022. Https://Doi.Org/10.18502/Cmm.8.1.9210.

[21] Rajni E, Chaudhary P, Garg Vk, Sharma R, Malik M. A Complete Clinico-Epidemiological And Microbiological Profile Of Candidemia Cases In A Tertiary-Care Hospital In Western India. Antimicrobial Stewardship & Healthcare Epidemiology 2022;2:E37. Https://Doi.Org/10.1017/Ash.2021.235.

[22] Chander J, Singla N, Sidhu Sk, Gombar S. Epidemiology Of Candida Blood Stream Infections: Experience Of A Tertiary Care Centre In North India. The Journal Of Infection In Developing Countries 2013;7:670–5. Https://Doi.Org/10.3855/Jidc.2623.

[23] Tak V, Mathur P, Varghese P, Gunjiyal J, Xess I, Misra Mc. The Epidemiological Profile Of Candidemia At An Indian Trauma Care Center. J Lab Physicians 2014;6:096–101. Https://Doi.Org/10.4103/0974-2727.141506.

[24] Dermitzaki N, Baltogianni M, Tsekoura E, Giapros V. Invasive Candida Infections In Neonatal Intensive Care Units: Risk Factors And New Insights In Prevention. Pathogens 2024;13:660. Https://Doi.Org/10.3390/Pathogens13080660.

[25] Intra J, Sala Mr, Brambilla P, Carcione D, Leoni V. Prevalence And Species Distribution Of Microorganisms Isolated Among Non-Pregnant Women Affected By Vulvovaginal Candidiasis: A Retrospective Study Over A 20 Year-Period. Journal Of Medical Mycology 2022;32:101278. Https://Doi.Org/10.1016/J.Mycmed.2022.101278.

[26] Bitew A, Abebaw Y. Vulvovaginal Candidiasis: Species Distribution Of Candida And Their Antifungal Susceptibility Pattern. Bmc Womens Health 2018;18:94. Https://Doi.Org/10.1186/S12905-018-0607-Z.

[27] Lockhart Sr, Etienne Ka, Vallabhaneni S, Farooqi J, Chowdhary A, Govender Np, Et Al. Simultaneous Emergence Of Multidrug-Resistant Candida Auris On 3 Continents Confirmed By Whole-Genome Sequencing And Epidemiological Analyses. Clinical Infectious Diseases 2017;64:134–40. Https://Doi.Org/10.1093/Cid/Ciw691.

[28] Grossman Nt, Chiller Tm, Lockhart Sr. Epidemiology Of Echinocandin Resistance In Candida. Curr Fungal Infect Rep 2014;8:243–8. Https://Doi.Org/10.1007/S12281-014-0209-7.

[29] Deorukhkar Sc, Saini S, Mathew S. Virulence Factors Contributing To Pathogenicity Of Candida Tropicalis And Its Antifungal Susceptibility Profile. Int J Microbiol 2014;2014:1–6. Https://Doi.Org/10.1155/2014/456878.

[30] Bairwa G, Hee Jung W, Kronstad Jw. Iron Acquisition In Fungal Pathogens Of Humans. Metallomics 2017;9:215–27. Https://Doi.Org/10.1039/C6mt00301j.

[31] Knight Sab, Vilaire G, Lesuisse E, Dancis A. Iron Acquisition From Transferrin By Candida Albicans Depends On The Reductive Pathway. Infect Immun 2005;73:5482–92. Https://Doi.Org/10.1128/Iai.73.9.5482-5492.2005.

[32] Almeida Rs, Wilson D, Hube B. Candida Albicans Iron Acquisition Within The Host. Fems Yeast Res 2009;9:1000–12. Https://Doi.Org/10.1111/J.1567-1364.2009.00570.X.

[33] Hiller D, Sanglard D, MorschhäUser J. Overexpression Of The Mdr1 Gene Is Sufficient To Confer Increased Resistance To Toxic Compounds In Candida Albicans. Antimicrob Agents Chemother 2006;50:1365–71. Https://Doi.Org/10.1128/Aac.50.4.1365-1371.2006.

[34] Bhattacharya S, Sobel Jd, White Tc. A Combination Fluorescence Assay Demonstrates Increased Efflux Pump Activity As A Resistance Mechanism In Azole-Resistant Vaginal Candida Albicans Isolates. Antimicrob Agents Chemother 2016;60:5858–66. Https://Doi.Org/10.1128/Aac.01252-16.

[35] Morschhäuser J, Barker Ks, Liu Tt, Blaß-Warmuth J, Homayouni R, Rogers Pd. The Transcription Factor Mrr1p Controls Expression Of The Mdr1 Efflux Pump And Mediates Multidrug Resistance In Candida Albicans. Plos Pathog 2007;3:E164. Https://Doi.Org/10.1371/Journal.Ppat.0030164.

[36] Jahanshiri Z, Manifar S, Arastehnazar F, Hatami F, Lotfali E. Azole Resistance In Candida Albicans Isolates From Oropharyngeal Candidiasis Is Associated With Erg11 Mutation And Efflux Overexpression. Jundishapur J Microbiol 2022;15. Https://Doi.Org/10.5812/Jjm-131046.

[37] You L, Qian W, Yang Q, Mao L, Zhu L, Huang X, Et Al. Erg11 Gene Mutations And Mdr1 Upregulation Confer Pan-Azole Resistance In Candida Tropicalis Causing Disseminated

Candidiasis In An Acute Lymphoblastic Leukemia Patient On Posaconazole Prophylaxis. Antimicrob Agents Chemother 2017;61. Https://Doi.Org/10.1128/Aac.02496-16.

[38] Jin L, Cao Z, Wang Q, Wang Y, Wang X, Chen H, Et Al. Mdr1 Overexpression Combined With Erg11 Mutations Induce High-Level Fluconazole Resistance In Candida Tropicalis Clinical Isolates. Bmc Infect Dis 2018;18:162. Https://Doi.Org/10.1186/S12879-018-3082-0.

[39] Pandey N, Tripathi M, Gupta Mk, Tilak R. Overexpression Of Efflux Pump Transporter Genes And Mutations In Erg11 Pave The Way To Fluconazole Resistance In Candida Tropicalis: A Study From A North India Region. J Glob Antimicrob Resist 2020;22:374–8. Https://Doi.Org/10.1016/J.Jgar.2020.02.010.

[40] Rojas Ae, Cárdenas Ly, García Mc, Pérez Je. Expression Of Erg11, Erg3, Mdr1 And Cdr1 Genes In Candida Tropicalis. Biomédica 2023;43:144–55. Https://Doi.Org/10.7705/Biomedica.6852.