Research Article

Epidemiology and Virulence Determinants including Biofilm Profile of *Candida* Infections in an ICU in a Tertiary Hospital in India

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The purpose of this prospective study was to isolate, speciate, and determine antifungal susceptibility and virulence patterns of *Candida* species recovered from the intensive care units (ICUs) in an Indian hospital. Study included 125 medical/postoperative patients admitted to ICU. Identification and speciation of yeast isolates were done by the biochemical methods. Antifungal susceptibility was done by broth microdilution method. Virulence testing of *Candida* species was done by phospholipase, proteinase, and adherence assay. A total of 103 *Candida* isolates were isolated; *C. tropicalis* was the predominant species (40.7%), followed by *C. albicans* (38.83 %), *C. glabrata* (11.65%), *C. parapsilosis* (3.88%), and 1.94% each of *C. krusei*, *C. kefyr*, and *C. sphaerica*. 60 *Candida* isolates (58.25%) showed resistance to fluconazole, while 7 (6.7%) isolates showed resistance to amphotericin B. Phospholipase and proteinase activities were seen in 73.8% and 55.3% *Candida* isolates with different species showing a wide range of activities, while 68.9% *Candida* isolates showed 4+ adherence activity. The present study revealed that *nonalbicans Candida* species (NAC spp.) caused most of the cases of Candidemia in the ICU patients. The isolation of *C. tropicalis* from a large number of cases highlights the ability of this pathogen to cause bloodstream infections. The presence of azole resistance is a matter of concern.

1. Introduction

Fungal infections are emerging as a problem of significant magnitude in hospitals during the last decade, especially in the ICUs, which are epicenters for infections such as Candidemia and invasive *Candida* infection (ICI). The escalating problem in ICUs is probably due to an increasing population of immunocompromised patients. A survey of the epidemiology of sepsis conducted in USA revealed that the incidence of fungal sepsis increased threefold between 1979 and 2000 [1]. Fungal infections account for nearly 8% of all nosocomial infections; *Candida* is the responsible agent in 80% of the cases [2]. *Candida* species are approximately the fourth most common cause of nosocomial infections in ICUs, according to data from the National Nosocomial

Infections Surveillance System and the European Prevalence of Infection in Intensive Care [3].

Critically ill patients who are treated in the ICUs are very susceptible to infections due to acquired defects in host defense mechanisms from the immunosuppressive effect of the underlying disease, recent surgery, trauma, and concurrent drug therapy. Infections occur in 15–40% of all ICU admissions and central venous catheters are considered as the most common risk factor for the development of Candidemia in these patients [4, 5]. The *Candida* biofilm results in antifungal drug resistance and protection of the fungus from host defenses [6], which carry important clinical repercussions.

Candida albicans has historically been the most frequent cause of Candidiasis. During recent decades, several

countries around the world have witnessed a change in the epidemiology of *Candida* infections, characterized by a progressive shift from a predominance of *Candida albicans* toward a predominance of NAC spp. (including *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*) [1].

In order to get an insight into the present scenario of ICU in an Indian hospital, in the present prospective study, an attempt was made to isolate and speciate the *Candida* species from various clinical samples and to know their *in vitro* pattern of susceptibility to fluconazole and amphotericin B and virulence patterns.

2. Materials and Methods

The present study was conducted from 2006 to 2008 at the Department of Microbiology, Maulana Azad Medical College and Associated Lok Nayak Hospitals, New Delhi, India, which is a 1500-bedded tertiary care hospital. One hundred and twenty-five medical/postoperative patients admitted to ICU for >48 hours undergoing therapy for one or more acute organ system failure or requiring intensive postoperative monitoring were studied. A total of 356 samples including blood, urine, tracheal aspirate, urinary catheter, endotracheal tube, and abdominal drains were collected and processed from the patients. Cases that had antifungal drug exposure during hospitalization were excluded from the study. All samples were inoculated on Sabouraud dextrose agar slants containing gentamicin (0.02 mg/mL) and cycloheximide (0.5%). One set of inoculated slants was incubated at 25°C and the other at 37°C, and they were examined on alternate days for the growth up to 4–6 weeks.

Identification and speciation of the isolates were conducted by colony morphology, gram staining, germ tube formation, corn meal agar with Tween80, HiCrome *Candida* agar, and an enzymatic triphenyltetrazolium chloride reduction; for further characterization, each isolate was subjected to carbohydrate assimilation and fermentation tests as per standard recommended procedures [7].

The *in vitro* minimal inhibitory concentrations (MICs) of the compounds were determined by the broth microdilution method as per Clinical and Laboratory Standards Institute (CLSI) M27-A3 document using RPMI medium and MOPS buffer. The concentration ranges tested were 0.125–128 μg/mL for fluconazole and 0.016–16 μg/mL for amphotericin B. The quality control was performed by testing *C. parapsilosis* (ATCC22019) and *C. krusei* (ATCC6258) from the American Type Culture Collection (ATCC) with each batch of clinical isolates [8]. The MIC breakpoints recommended by CLSI guidelines were followed (CLSI, 2008). For fluconazole, MIC breakpoints were as follows: sensitive, MIC $\leq$ 8 μg/mL; susceptible-dose dependent (SDD), MIC 16–32 μg/mL; resistant, MIC $\geq$ 64 μg/mL. For amphotericin B, isolates with MICs of $\geq$ 1 μg/mL were categorized as resistant (CLSI, 2008).

Candida proteinase was detected by the Mohandas and Ballal method [9] using bovine serum albumin medium. The proteinase activity (Pz) was determined in terms of the ratio of the diameter of the well to the diameter of the proteolytic zone [9]. The extracellular phospholipase activity

of the isolates was screened by growing them on egg-yolk agar and measuring the size of the zone of precipitation by the Samaranayake et al. method [10]. Biofilm-formation was determined spectrophotometrically by the Branchini et al. method [11].

3. Results

The patients belonged to a wide age group from 13 years to 90 years with a mean age of 33 years. Out of the 125 study cases, 53 (42.4%) belonged to 16–30 years age group. Males were predominant with a male to female ratio of 1.2 : 1. In our study 86.29% patients were admitted to ICU for less than 1 week followed by 9.68% for 1-2 weeks, while very few patients (6%) had a stay of more than 2-3 weeks. The most common organ system involved in our patients was gastrointestinal system (27.2% cases) followed by central nervous system (19.4% cases) and respiratory system (19% cases). Diabetes mellitus was seen in 14.5% cases, while fever, multiorgan system involvement, and infection were seen in 37.5%, 17.5%, and 16% cases, respectively.

A total of 356 patient samples were collected. They included blood in 125 cases (100%), urine in 112 cases (89.6%), tracheal aspirate in 88 cases (70.4%), urinary catheter in 18 cases (14.4%), endotracheal tube in 10 cases (8%), and abdominal drains in 3 cases (2.4%). The most common risk factor found in our patients was that of presence of indwelling device in 100 patients followed by prolonged antibiotic therapy in 78 patients and surgery in 73 patients. Prolonged antibiotic therapy was also seen in Candidemia cases as well as *Candida* colonization cases (Table 1).

A total of 103 *Candida* isolates were obtained on culture. The majority (39.8%) of the culture isolates were obtained from urine samples followed by tracheal aspirate (28.2%), urinary catheter (10.7%), blood (9.7%), endotracheal tube (6.8%), and abdominal drains (2.9%). *C. tropicalis* (40.8%) and *C. albicans* (38.8 %) were the most common *Candida* species causing infections in patients, followed by *C. glabrata* (10.7%), *C. parapsilosis* (3.9%), *C. kefyr* (1.9%), *C. krusei* (1.9%), and *C. sphaerica* (1.9%). *C. albicans* (4.8%), *C. tropicalis* (3.9%) and *C. glabrata* (0.9%) were the organisms incriminated in causation of candidemia (Table 2).

C. tropicalis was most often isolated from urine (35.71%) followed by tracheal aspirate (26.19%), urinary catheter (14.28%), endotracheal tube (11.90%), blood (09.52%), abdominal drains (2.38%), and sputum (0%). *C. albicans* was more often isolated from urine (42.50%) and tracheal aspirate (32.50%). However, *C. parapsilosis*, *C. krusei*, *C. kefyr*, and *C. sphaerica* were isolated from urine, urinary catheter, and tracheal aspirate (Table 2).

Out of the 103 *Candida* isolates, 6.7% isolates showed resistance to amphotericin B, while 60 (58.25%) showed resistance to fluconazole. The results of Table 2 showed that 67.5% *C. albicans* were resistant to fluconazole followed by 57.14% *C. tropicalis*, 50% each of *C. parapsilosis* and *C. sphaerica*, and 45.4% *C. glabrata,* while a lower resistance to amphotericin B of 4.7%, 5%, and 9% was seen by *C. tropicalis*, *C. albicans*, and *C. glabrata*, respectively. The blood stream

TABLE 1: Analysis of potential risk factors found for *Candida* infection in ICU patients.

Continuous variables (mean ± SD)	Controls[a] (n = 70, 56%)	Candidemia (n = 10, 8.7%)	P value	Candidiasis/colonization (n = 45, 36%)	P value
Age (years)	36 ± 16.4	37 ± 17.6	0.85	37 ± 16.56	0.75
Length of hospital stay (days)	9.7 ± 13.8	7.8 ± 4.7	0.66	9.4 ± 13.9	0.99
Categorical variables (number)	Controls[a] (%) n = 70	Candidemia (%) n = 10	P value	Candidiasis/colonization (%) n = 45	P value
Surgery (73)	42 (60%)	6 (60%)	1	27 (67.5%)	1
Trauma (28)	18 (11.4%)	2 (20%)	0.69	9 (20%)	0.63
Pneumonia (17)	08 (11.4%)	3 (30%)	0.2	9 (20%)	0.32
Neurological (12)	08 (11.4%)	0 (0)	NA	4 (8.9%)	0.90
Malignancy (9)	5 (7.14%)	1 (10%)	0.5	4 (8.9%)	0.73
TB (10)	3 (4.28%)	0 (0)	NA	5 (11.1%)	0.25
Renal failure (3)	0 (0)	0 (0)	NA	3 (6.67%)	NA
Heart disease (2)	1 (1.42%)	0 (0)	NA	1 (2.2%)	1
Anemia (1)	1 (1.42%)	0 (0)	NA	0 (0)	NA
Burns (1)	1 (1.42%)	0 (0)	NA	0 (0)	NA
Peritonitis (23)	09 (12.85%)	1 (10%)	1	12 (26.67%)	**0.019**
Multiple organ (18) system involvement	3 (4.28%)	1 (10%)	0.4	15 (33.3%)	**0.001**
Indwelling devices (100)	35 (50%)	10 (100%)	NA	45 (100%)	NA
Prolonged antibiotic therapy (78)	40 (57.14%)	8 (80%)	0.3	33 (73%)	0.08
Duration of stay >1 week in ICU (34)	11 (15.71%)	3 (30%)	0.5	21 (46.6%)	**0.005**
Use of immunosuppressants (32)	13 (18.57%)	2 (20%)	0.9	18 (40%)	**0.020**
Diabetes mellitus (25)	10 (14.28%)	4 (40%)	0.11	14 (31.1%)	0.05
Smoking (15)	9 (12.85%)	2 (20%)	0.9	6 (13.3%)	0.9

Controls[a]: patients admitted to the same ICU with no Candidemia.
Significant P values ($P \leq 0.05$) are indicated in bold. NA: not applicable.

TABLE 2: Profile of *Candida* species isolated from different clinical samples.

Sample	C. albicans no. (%)	C. tropicalis no. (%)	C. krusei no. (%)	C. parapsilosis no. (%)	C. kefyr no. (%)	C. sphaerica no. (%)	C. glabrata no. (%)	Total no. (%)
Blood	05 (4.8%)	04 (3.9%)	0	0	0	0	01 (0.9%)	10 (9.7%)
Urine	17 (16.5%)	15 (14.6%)	1 (0.9%)	2 (1.9%)	1 (0.9%)	1 (0.9%)	4 (3.9%)	41 (39.8%)
Sputum	0	0	0	0	0	0	2 (1.9%)	2 (1.9%)
Urinary catheter	3 (2.9%)	6 (5.8%)	0	1 (0.9%)	0	1 (0.9%)	0	11 (10.7%)
Endotracheal tube	1 (0.9%)	5 (4.8%)	0	0	0	0	1 (0.9%)	7 (6.8%)
Tracheal aspirate	13 (12.6%)	11 (10.7%)	1 (0.9%)	0	1 (0.9%)	0	3 (2.9%)	29 (28.2%)
Abdominal drains	1 (0.9%)	1 (0.9%)	0	1 (0.9%)	0	0	0	3 (2.9%)
Total	40 (38.8%)	42 (40.8%)	2 (1.9%)	4 (3.9%)	2 (1.9%)	2 (1.9%)	11 (10.7%)	103 (100%)

isolates of *C. albicans* and *C. tropicalis* showed a resistance of 40% and 25%, respectively, to fluconazole in contrast to the urinary and the indwelling isolates which were much more resistant (Table 4).

Phospholipase activity was detected in 73.78% isolates. Out of 73.78% phospholipase positive isolates, 71.05% isolates were NAC spp. and 28.95% were *C. albicans*. Among NAC spp., *C. tropicalis* (64.81%) was the highest phospholipase producer followed by *C. glabrata* (20.37%), whereas *C. kefyr* (1.85%) was least producer. The proteinase activity was seen in 55.33% *Candida* isolates. Out of 55.33% proteinase positive isolates, 57.89% were NAC spp. and 42.10% were *C. albicans*.

TABLE 3: The *in vitro* antifungal susceptibilities of *Candida* isolates to fluconazole and amphotericin B.

| Species (no. of isolates) | Antifungal drug susceptibility pattern | | | | |
| | Fluconazole | | | Amphotericin B | |
	S[a] (≤8 μg/mL) no. (%)	SDD[b] (16–32 μg/mL) no. (%)	R[c] (≥64 μg/mL) no. (%)	S[a] (≤1 μg/mL) no. (%)	R[c] (≥1 μg/mL) no. (%)
C. albicans (40)	10 (25%)	3 (7.5%)	27 (67.5%)	38 (95%)	2 (5%)
C. tropicalis (42)	15 (35.7%)	3 (7.1%)	24 (57.2%)	40 (95.2%)	2 (4.8%)
C. glabrata (11)	6 (54.5%)	0	5 (45.5%)	10 (90.9%)	1 (9.1%)
C. sphaerica (02)	1 (50%)	0	1 (50%)	2 (100%)	0
C. parapsilosis (04)	2 (50%)	0	2 (50%)	4 (100%)	0
C. kefyr (02)	2 (100%)	0	0	2 (100%)	0
C. krusei (02)	0	1 (50%)	1 (50%)	0	2 (100%)

S[a]: susceptible range.
SDD[b]: susceptible dose-dependent range.
R[c]: resistant range.

Among NAC spp., *C. tropicalis* (66.66%) was the highest proteinase producer followed by *C. glabrata* (12.12%), whereas *C. kefyr* showed no proteinase activity. The adherence activity was shown by 99.02% *Candida* isolates; out of these isolates 10.78% were weak positive {2+} and 19.60% strong positive {3+}, while 69.61% isolates showed maximum adherent activity {4+}. NAC species (60.78%) produced biofilm in comparison to *C. albicans* (39.22%) showing relatively lower slime production. Also strong biofilm production was seen in cases of *C. tropicalis* compared to weak biofilm production that was seen in *C. kefyr*, *C. krusei*, and *C. sphaerica*.

4. Discussion

Patients in ICU are at a higher risk of acquiring nosocomial infections compared with patients in general wards due to the severity of the underlying illnesses and iatrogenic factors related to the high frequency of invasive procedures needed for the monitoring and treatment [12] which include insertion of intravascular catheters, endotracheal intubation, and positive pressure ventilation, urinary catheterization and surgical operations.

Studies from different parts of the world on nosocomial infections have shown that patient and treatment factors are risk factors for the development of nosocomial infections. Some identified risk factors related to treatment have been length of hospital or ICU stay, presence of indwelling devices, and intake of drugs like broad spectrum antibiotics and immunosuppressants [13] which were also seen in our cases as 86.29% patients were admitted to ICU for less than 1 week followed by 9.68% for 1-2 weeks. According to Sydnor and Perl, admission to ICU itself was a significant risk factor; roughly 25% of nosocomial infections occur in intensive care units (ICUs), which have been estimated to increase ICU length of stay by 4.3 to 15.6 days [14]. Length of stay in the ICU is also associated with increased risk for *Candida* infections, which rises rapidly after 7–10 days [1]. History of smoking was elicited in 26% of cases corroborated by a recent study from Mumbai also showing the association of nosocomial pneumonia and mortality with tobacco

smoking [15]. Workers from Delhi have reported a higher risk of nosocomial infections in ICUs attributed to the severity of the underlying illness and the development of iatrogenic factors related to the high frequency of invasive procedures required in these patients [12].

Peritonitis, multiple organ system involvement, duration of stay >1 week, and immunosuppressant were significantly associated with the group with *Candida* colonization ($P \leq 0.05$) unlike the Candidemia cases. The number of cases of Candidemia was may be too low to get a significant association. Similar association with antibiotic and immuno-suppressant therapy along with catheterization and surgery in causation of nosocomial Candidiasis has been quoted in other studies in North India [16, 17].

In ICU patients, the most common types of *Candida* infections are seen to comprise bloodstream, catheter-related, intra-abdominal, and urinary tract infections [6]. In our patients, culture isolations were obtained in 39.8% of urine samples followed by 31.06% of tracheal aspirate in addition to a varying percentage from other samples like urinary catheter (10.6%), blood (8.7%), endotracheal tube (6.7%), and abdominal drains samples (2.9%). Different studies reported the predominance of *C. albicans* as a leading cause of invasive candidiasis. However, recently, several countries around the world have witnessed a change in the epidemiology of *Candida* infections, characterized by a progressive shift from a predominance of *C. albicans* toward a predominance of NAC species [1]. Although *C. albicans* remains the most commonly isolated species from cases of Candidemia in USA, Europe, and South America (Brazil), its dominance is slowly giving way to an increase in NAC species such as *C. glabrata* [18].

However, in India, *C. tropicalis* appears to be emerging as the most common cause of Candidemia [18]. 50% of our cases of Candidemia were due to *C. albicans* followed by *C. tropicalis* (40%) and *C. glabrata* (10%). Findings of our study quite similar to the epidemiological data from the Indian subcontinent showed that 50% of our nosocomial candidemia cases were due to NAC spp. of which *C. tropicalis* was the most dominant [19].

Table 4: Sample wise *in vitro* antifungal resistance profile of *Candida* isolates to fluconazole and amphotericin B.

Sample	C. albicans			C. tropicalis			C. krusei			C. parapsilosis			C. kefyr			C. sphaerica			C. glabrata		
	FLU		AMB	FLU		AMB	FLU		AMB	FLU		AMB	FLU		AMB	FLU		AMB	FLU		AMB
	SDD^b	R^c	R^c	SDD^b	R^c	R^c	SDD^b	R^c	R^c	SDD^b	R^c	R^c	SDD^b	R^c	R^c	SDD^b	R^c	R^c	SDD^b	R^c	R^c
	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)	no. (%)
Blood	0	2 (40)	0	0	1 (25)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (100)	0
Urine	3 (17.6)	12 (70.6)	2 (11.8)	0	9 (60)	0	0	1 (100)	1 (100)	0	0	0	0	0	0	0	1 (100)	0	0	1 (25)	0
Sputum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (50)	0
Urinary catheter	0	3 (100)	0	2 (33.3)	2 (33.3)	0	0	0	0	0	1 (100)	0	0	0	0	0	0	0	0	0	0
Endotracheal tube	0	0	0	0	4 (80)	4 (80)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tracheal aspirate	0	10 (76.9)	0	1 (9.1)	7 (63.6)	1 (9.1)	1 (100)	0	1 (100)	0	0	0	0	0	0	0	0	0	0	2 (66.7)	1 (33.3)
Abdominal drains	0	0	0	0	1 (100)	0	0	0	0	0	1 (100)	0	0	0	0	0	0	0	0	0	0
Total	3 (7.5)	27 (67.5)	2 (5)	3 (7.1)	24 (57.1)	5 (11.9)	1 (50)	1 (50)	2 (100)	0	2 (50)	0	0	0	0	0	1 (50)	0	0	5 (45.4)	1 (9.0)

SDD^b: susceptible dose-dependent range.
R^c: resistant range.
FLU: fluconazole drug.
AMB: amphotericin B drug.

Interestingly, in our study, *C. tropicalis* was most often isolated from urine (35.71%) followed by tracheal aspirate (26.19%), urinary catheter (14.28%), endotracheal tube (11.90%), blood (09.52%), abdominal drains (2.38%), and sputum (0%). *C. albicans* was more often isolated from urine (42.50%) and tracheal aspirate (32.50%). However, *C. parapsilosis*, *C. krusei*, *C. kefyr*, and *C. sphaerica* were isolated only from urine, urinary catheter, and tracheal aspirate. 36.9% of isolates of *C. tropicalis* were from colonization sites (Table 2).

Some of these emerging species have been correlated with increased virulence. An increasing role for NAC spp. was also noticed in studies performed among ICU patients, although the issue is somewhat controversial [20]. The increased use of fluconazole has been seen to be associated with the predominance of many NAC spp., especially *C. tropicalis* species[21]. Findings in our study have confirmed and corroborated the earlier reports of increased resistance to fluconazole among isolates of *C. tropicalis* from the Asia-Pacific region [22]. Also *C. parapsilosis*, *C. krusei*, *C. kefyr*, and *C. sphaerica* have shown a predilection towards isolation from urinary catheters and tracheal aspirates in our cases, explaining their role in inherent biofilm formation leading to difficulty in eradication from the site and development of resistance in their long stay. Several investigators have postulated that the widespread use of fluconazole would have a big role in selecting out yeast species intrinsically resistant or less sensitive to fluconazole, such as *C. krusei*, *C. glabrata*, or *C. tropicalis* [20]. In our study, a large proportion of the *C. tropicalis* (57.14%, 24/42) was resistant to fluconazole; 90.4% of *C. tropicalis* isolates were responsible for *Candida* colonization with many of them (50%) being involved in biofilm formation. Punithavathy et al. [23] from South India, however, reported that (22%, 11/50) isolates of *C. tropicalis* were resistant to fluconazole.

Blood stream isolates of *C. albicans*, *C. tropicalis* were 40% and 25% while the urinary isolates were 70.6% and 60% resistant to fluconazole respectively, in comparison to a higher resistance going up to 100% seen in isolates of endotracheal tubes, tracheal aspirate and abdominal drains. Our results of 25% resistance to fluconazole in blood stream isolates of *C. tropicalis* were consistent with a report from Maharashtra and Delhi, in which 37% and up to 83%, respectively, were resistant to fluconazole [24, 25], while the isolates from urine and indwelling devices showed a higher resistance quite like a similar picture being seen in Chennai, South India, reflecting the high level of resistance in colonized isolates involved in biofilm formation predisposing to candidemia [26].

Candida glabrata has also become important because of its increasing incidence worldwide; it was seen in 11.6% of our study cases with 10 out 11 isolates from colonization sites exhibiting a high resistance to azoles. *C. krusei*, found in 2 cases again from colonization sites in our study, is another important *Candida* species which is gaining clinical significance because of its intrinsic resistance toward azoles and poor susceptibility to all other antifungals, including amphotericin B [27]. *C. sphaerica* was isolated from urine and urinary catheter samples only and 50% of *C. sphaerica*

isolates were resistant to fluconazole, while no resistance to amphotericin B was seen. On the other hand both the isolates of *C. kefyr* from urine and tracheal aspirate were sensitive to fluconazole and amphotericin B. However, in a study in Turkey, all the *C. sphaerica* strains (3/3) were sensitive to Fluconazole and resistant to amphotericin B and 25% (2/8) *Candida kefyr* strains were susceptible dose dependent (SDD) and 37.5% (3/8) sensitive to fluconazole and 37.5% (3/8) were SDD and 25% (2/8) resistant to amphotericin B as reported by Abaci and Haliki-Uztan [28] (Table 3).

Virulence activity of *C. albicans* and *C. tropicalis* isolates from blood samples were 40% and 75% for phospholipase-activity, 60% and 25% for proteinase-activity and 100% each for adherent-activity, respectively. But the same was much more in the biofilm isolates from urinary catheter, endotracheal tube, and tracheal aspirate: 33.3% to 100% for phospholipase activity, 61.5% to 100% for proteinase activity, and 100% adherent activity in the urinary catheter, endotracheal tube and tracheal aspirate going up to 100% phospholipase activity, proteinase activity, and adherent activity in abdominal drains.

The proteinase-producing capacity of *C. albicans* (42.10%) was less than that of NAC spp. (57.89%) in our patients, whereas workers from Manipal showed NAC spp. (50.45%) to be lower proteinase producers than *C. albicans* (67.34%) [29]. In our study among NAC spp. *C. tropicalis* (66.66%) was the highest proteinase producer, whereas *C. kefyr* showed no proteinase activity consistent with studies of Al-Nedaithy [30] which showed *C. tropicalis* (95%) to be proteinase producer and no activity with *C. kefyr*. 50–100% of our *Candida* isolates showed phospholipase activity similar to the previous studies conducted by Price et al. [31] and Wu et al. [32] who reported phospholipase activity in 30–100% of their *Candida* isolates from various groups of patients and from various sites. Also a total of 22 (28.95%) of *C. albicans* isolates and 54 (85.7%) of NAC spp. isolates produced phospholipase in our cases, while Mohandas and Ballal [29] from Manipal and Ibrahim et al. [33] reported 23 (46.93%) of their *C. albicans* isolates and 26 (42%) of NAC spp. isolates producing phospholipase with greater extracellular phospholipase activity. In our study among NAC spp., *C. tropicalis* (64.81%) was the highest phospholipase producer concordant with studies of Negri et al. [34] and Júnior Jr. et al. [35] showing *C. tropicalis* to be the species with the highest number of positive isolates for phospholipase activity (up to 91.7%).

Biofilm production has been seen to promote persistence of infection as was evidenced in *Candida* isolates of OPC patient in a major medical center in India [36]. Our study showed that 60.78% of isolates of NAC spp. produced biofilm in comparison to 39.21% of *C. albicans* producing lower slime (Figure 1). Shin et al. [5] and Pathak et al. [37] also reported higher biofilm-forming ability of NAC spp. than *C. albicans* species. Our data also suggest the production of stronger biofilms by *C. tropicalis* (40.19%) followed by *C. albicans* (39.21%). Weak biofilm production was seen with *C. kefyr*, *C. krusei*, and *C. sphaerica* (1.96% each). Biofilm positivity has also been seen to occur most frequently in *C. tropicalis* isolates followed by *C. parapsilosis*, *C. glabrata*,
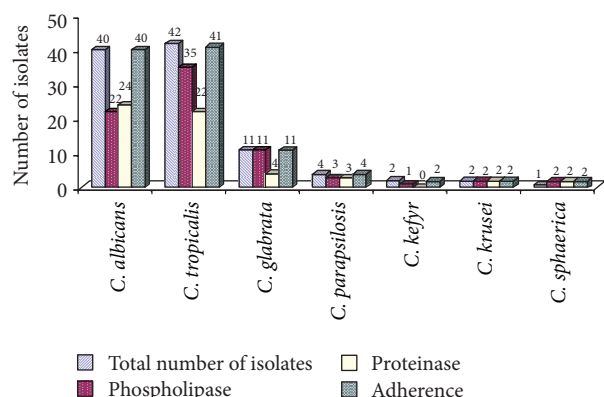
FIGURE 1: Pathogenicity profile of *Candida* species isolates; NAC spp. had greater biofilm forming ability in comparison to *Candida albicans* species.

and *C. albicans* [5]. Kumar et al. [38] also reported *C. tropicalis* to be strongly positive for biofilm production. However, Hawser and Douglas [39] reported that isolates of *C. parapsilosis* and *C. glabrata* were significantly less likely to produce biofilms than the more pathogenic *C. albicans*. Biofilm may be a specific trait of a *Candida* that is not associated with differences in adherence but with slowness of growth; however, *Candida* biofilms contribute both to the pathogenesis of superficial and systemic Candidiasis as they are notoriously resistant to antifungal drugs [36].

Advances in modern medicine and patient management have seen increased use of prosthetic biomaterials. This has led to the complication associated with biofilm formation on these materials, the constituent organisms of which are different in their growth rate and antifungal susceptibility as compared to isolates not associated with biofilms. The knowledge of the local epidemiological trends in the *Candida* species isolated is important to guide therapeutic choices. Our study demonstrates a shift of *C. albicans* predominance towards a NAC spp. in causation of candidiasis among ICU population with the isolation of *C. tropicalis* from a large number of cases highlighting the importance of this pathogen. The presence of high resistance to fluconazole by *C. albicans* followed by *C. tropicalis* is another important finding in our study and may have important clinical repercussions. It was seen that NAC spp. had greater biofilm-forming ability when compared to *C. albicans* species especially *C. tropicalis* showed the highest ability to produce phospholipase, proteinase, and biofilms. Thus in the light of virtual absence of such data from our area, this study would help in understanding the properties of the biofilms, with respect to growth characteristics and drug sensitivity, and should be a great help in understanding the present trends and predicting the overall long-term trends that will be helpful to physicians and to antibiotic use committees in establishing guidelines for the appropriate use of antifungal agents. This will help in reducing complications associated with biofilm formation and devise control strategies and consequently reducing morbidity, mortality, and treatment costs. The results obtained here open perspectives of new investigations that are aimed at establishing and broadening knowledge of understanding relationship between strain types and properties such as pathogenicity, commensality, and infectivity, an important interplay in host-pathogen relationship specially as far as *Candida* infections are concerned.

Conflict of Interests

The authors declare that they have no conflict of interests.

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