Section: Microbiology

Original Research Article

ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CLINICAL ISOLATES OF CANDIDA FROM A TERTIARY CARE HOSPITAL IN KARNATAKA, INDIA

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ABSTRACT

Background: The present study was conducted to isolate & identify the species and to know the antifungal susceptibility pattern of the isolated *Candida* species from different clinical specimens received in laboratory of microbiology at our tertiary care hospital. **Materials and Methods:** This study was a prospective study conducted by Department of Microbiology, at a tertiary care hospital in Karnataka, from January 2024 to December 2024. Total 100 *Candida* species were isolated from the suspected *candida* infection cases from different clinical specimens. *Candida* isolates were identified using standard microbiological procedures and speciation was done following conventional and HiCrome differential media. Antifungal susceptibility testing was done by disk diffusion method determined using Clinical and Laboratory Standards Institute (CLSI) guidelines. **Result:** Among the 100 clinical specimens processed, urine samples were maximum (38), followed by sputum (21).Sample were received from patients of all age group but maximum samples were isolated from patient belonging to 21-30 years of age followed by patient belonging to >60 years of age. Most common risk factor was prolonged antibiotic therapy (18). The number of non-*albicans candida* species (53) exceeded the number of *candida albicans* (47). Isolated species were *C. albicans* (47), *C. tropicalis* (26), *C. glabrata* (12), *C. krusei* (6), *C.parapsilosis* (5), *C.dubliniensis* (3), *C. guilliermondii* (1). Among all the anti-fungal agents it was observed that sensitivity to Amphotericin B was 100% followed by Voriconazole (84%), Itraconazole (69%), ketoconazole (47%) and least sensitivity was shown by fluconazole (45%). **Conclusion:** There is a changing trend of rise in the incidence of non *albicans Candida* over *Candida albicans*. In order to restrict the empirical use of antifungal agent prompt speciation of *Candida* isolates is helpful. This will further help the clinicians with correct treatment options.

INTRODUCTION

Developments in diagnostic modalities and therapeutic options has contributed to invasive fungal infections and colonization in large population of immunocompromised patients and /or those hospitalized with serious underlying conditions. Among these risk groups, *Candida* species are the most important contributor to the morbidity and mortality of HAI caused due to fungal etiology. There has been increase in the rate of *Candida* infections in different clinical settings throughout the world [1]. In a recent study the incidence of Candidemia of 6.9 per 1,000 intensive care unit (ICU) patients was reported, of this 7.5% of ICU patients were receiving antifungal therapy.[1,2] Candidemia increases mortality rates in the range of 20-49%,[3,4] with many open questions regarding management of Candidemia remain unanswered.[2]

Candidiasis is an opportunistic infection occurring in presence of predisposing factors like extensive and prolonged administration of broad-spectrum antimicrobials, corticosteroids, immunosuppressive agents and cytotoxic drugs, diabetes mellitus, HIV, chronic renal failure, hemodialysis, renal transplantation or indwelling urinary catheter.[3] Members of genus *Candida* lead to primary or secondary infection are associated with vast clinical spectrum of human infections ranging from superficial infection of the skin, mucus membranes to life threatening candidemia and hospital-acquired infection. Candidiasis accounts for 66-80% of fungal all the infections.[1]

More than 17 different species are known to be the causative agents of human infection by genus *Candida*.[2] The most common species causing infections in humans was *Candida albicans* till recent years but during the past decade non-*albicans*

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candida species has emerged as a significant pathogen[4].Now more than 90% of invasive infections are caused by *C.albicans, C. glabrata, C.parapsilosis, C.tropicalis and C.krusei*.[2,5]

Increased incidence of antifungal drug resistance is major cause of concern in management of Candidemia.[5] Intrinsic and emerging resistance to azoles group of antifungals represents a major challenge for prophylactic, therapeutic and empirical treatment options. This changing scenario has demanded routine antifungal susceptibility testing as both in-vitro resistance and toxicity issues must be considered during selecting an antifungal agent for the treatment.[6] The clinical importance of species-level identification is that each species of *Candida* differs in expression of putative virulence factors and antifungal susceptibility.[7]

Aims & objectives:

1. To isolate & identify *Candida* species from various clinical specimens.
2. To find out the Antifungal susceptibility pattern of the *Candida* species isolated.

The extensive use of antifungals for prophylaxis became the leading cause of colonization of Non-*albicans Candida* (NAC) species and increasing resistance to antifungal drugs. Changing etiology of candidiasis and emerging antifungal resistance necessitates early identification, speciation and antifungal susceptibility testing to select the appropriate antifungal agent to prevent the treatment failure.[1]

This study was taken up with the objective of generating data on different species of *Candida* obtained from different clinical samples, their characterization up to the species level and to determine their antifungal susceptibility pattern in these isolates that will help in early diagnosis & prompt therapeutic intervention.

MATERIALS AND METHODS

This prospective study was carried out in the department of Microbiology at a tertiary care center in Bangalore, Karnataka during the period January 2024- December 2024. Privacy and confidentiality were maintained in all cases.

Inclusion criteria:

Samples which isolated various candida species from different clinical specimens that were submitted to the Laboratory of Department of Microbiology at a tertiary care Centre, Bangalore from January 2024-December 2024.*Candida* species isolated obtained from blood, urine, sputum, Broncho-alveolar lavage, Tracheal aspirate, cerebrospinal fluid, pus, peritoneal fluid, high vaginal/ cervical swab, central line tip, nail clipping or skin scrapping were included in the study.

Exclusion criteria:

Samples that isolate the bacterial species only.

All the procedures were done according to standard operating procedures for the culture & sensitivity. Samples received in the microbiology laboratory was processed as soon as possible from the time of receiving. [10, 12]

Identification and Speciation of *Candida* Isolates:

Direct microscopy: Urine, sputum, oral swab, vaginal swab, nail clipping or skin scrapping were subjected to Potassium Hydroxide (KOH) wet mount for yeast cells with pseudohyphae. All the samples were subjected to gram stain to look for gram positive budding yeast-like cells with or without pseudohyphae, pus cells, epithelial cells or bacteria.

Culture: All the clinical samples that gave *Candida* growth on routine blood and macConkey agar were subcultured on Sabouraud's Dextrose Agar (SDA) and incubated at 25° C and 37°C. Blood culture samples collected in blood culture bottles were incubated in BacTAlert3D (Biomerieux, France) automated blood culture system and upon getting a positive alarm, were subcultured onto Sabouraud's dextrose agar (HiMedia, India) and blood agar plates after getting gram positive budding yeasts on gram stain of Blood culture broth.

On SDA, *Candida* produced creamy, smooth, pasty and convex colonies within 24-72 hours. Some species required more than three days to appear on culture medium.

Gram staining: Isolated colonies obtained on SDA were further subjected to gram staining to identify the budding yeast-like cells.

Urease test: Urease test was done to rule out Cryptococcus neoformans which is urease positive.

Criteria used to Indicate *Candida* Infection in Various Samples.

- Urine: Quantitative culture with colony count of $>10^5$ Colony Forming Unit (CFU)/ml of urine is associated with infection in patients without indwelling catheters and $>10^3$ CFU/ml for catheterized patients. Pyuria usually supports diagnosis of *Candida* infection. Low colony counts in presence of pyuria were considered significant. Repeat isolation in same patient was also considered significant.[10,13]
- Sputum: Considered acceptable on gram stain when 25 or more polymorphonuclear leukocytes were seen per low power field (10x) field with few (<10) squamous epithelial cells.[3]
- Blood: Candidemia is defined as presence of atleast one positive blood culture containing pure growth of *Candida* species with supportive clinical features.[10]
- Central venous tip: Greater than 15 CFU on roll plate culture was considered positive of Catheter-Related Bloodstream Infection (CRBSI).[11]
- Oral and vaginal swabs: Direct demonstration of pseudohyphae along with yeast-like cells using KOH wet mount or gram stain.[11]

Speciation- Conventional methods: Germ tube test, demonstration of chlamydospore formation on Cornmeal agar with Tween 80. [12-14]

HiCrome *Candida* differential agar: The *Candida* isolates were sub cultured on HiCrome *Candida*

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differential agar for species identification according to the manufacturer's instructions.[16]

1. *Candida albicans*: Light green coloured smooth colonies.
2. *Candida dubliniensis*: Dark green coloured smooth colonies.
3. *Candida tropicalis*: Blue to metallic blue coloured raised colonies.
4. *Candida glabrata:* Cream to white smooth colonies.
5. *Candida krusei*: Purple fuzzy colonies.
6. *Candida guilliermondii*: Light pink to pink colonies.
7. *Candida parapsilosis*: Light pink colonies.

Antifungal susceptibility testing: This was done by disk diffusion method according to CLSI (formerly NCCLS), 2009, M44-A2 guidelines using commercially available 6 mm antifungal discs (Himedia, Mumbai, India) such as fluconazole 25 µg, voriconazole 1 µg, amphotericin B 20 µg, itraconazole 10 µg and ketoconazole 30 µg.[17]

Due to the lack of defined breakpoints for itraconazole, ketoconazole and amphotericin B arbitrary values based on other studies and manufacturer (HIMEDIA, Mumbai) guidelines were employed.[3,11]

Interpretive Categories

Susceptible (S): The susceptible category implied that an infection due to the strain might be appropriately treated with the dose of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.

Susceptible-Dose Dependent (S-DD): The susceptible-dose dependent category included isolates with antifungal agent Minimum Inhibitory Concentration (MIC) that approached usually attainable blood and tissue levels and for which response rates might be lower than for susceptible isolates.

Resistant (R): This category included those resistant strains which were not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or when zone diameters had been in a range where clinical efficacy had not been reliable in treatment studies [Table 1].[3,11,17]

Table 1: Breakpoint zone diameter (mm) for Candida spp 11,17,5.

Antifungal agent	Disk content	Sensitive	Susceptible dose dependent	Resistant
Fluconazole	25 µg	>=19 mm	15-18 mm	<=14 mm
Voriconazole	1 µg	>=17 mm	14-16 mm	<=13 mm
Amphotericin B	20 µg	>=15 mm	13-14 mm	<=12 mm
Itraconazole	10 µg	>=17 mm	14-16 mm	<=13 mm
Ketoconazole	10 µg	>=28 mm	21-27 mm	<=20 mm

Quality control: Every batch of media prepared was checked for sterility by incubating at 37°C for 24 hours. Candida albicans American Type Culture Collection (ATCC) 90028 was used as quality control strain for the antifungal susceptibility testing.

Statistical analysis: will be done by Microsoft excel.

RESULTS

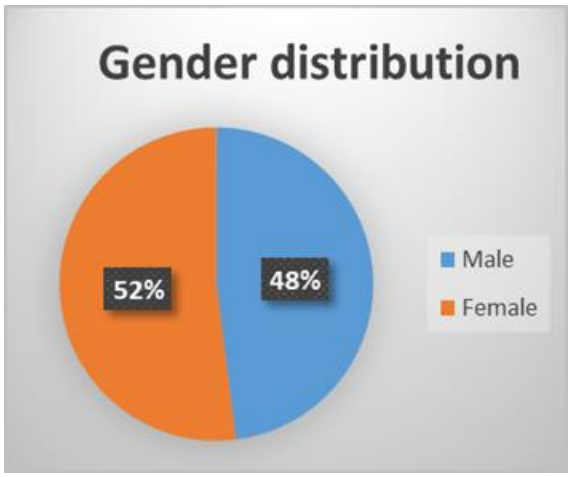

Figure 1: Gender distribution in isolated *Candida* species.

A total of 100 isolates of *Candida* species were obtained from different clinical specimens of patients visiting the Outpatient department (OPD) (30), admitted to In Patient Department (IPD) (38) and ICU (32) during the year Jan2024-Dec2024.Among these 100 isolates identified; 48 were from male patients and 52 from female patients [Figure 1]

Sample distribution according to age group was as follows, maximum samples were from age group 21-30 followed by ≥ 60 years age group [Figure 2].

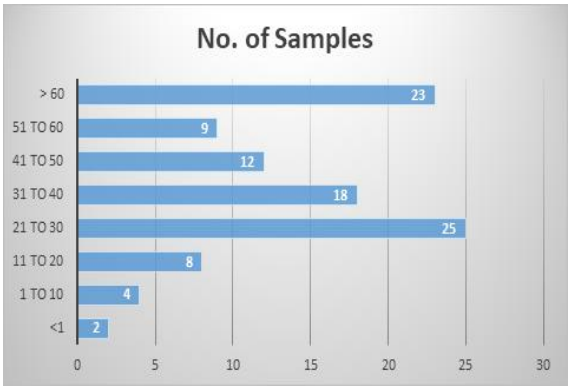

Figure 2: Distribution of *Candida* isolates among various age groups.

Out of 100 samples, urine samples were maximum that is 38, followed by sputum (21), pus (13), High Vaginal Swab(10), Oropharyngeal Swab (8), Blood(7), Endotracheal Aspirate(2) and Nail(1) [Table 2].

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Table 2: Distribution of candida isolates in various clinical samples.

Source	No. of Samples
Urine	38
Sputum	21
Pus	13
High Vaginal Swab	10
Oropharyngeal Swab	8
Blood	7
Endotracheal Aspirate	2
Nail	1

Most common risk factor was prolonged Antibiotic therapy (18). Other Risk factors associated were found to be prolonged hospital stay, use of corticosteroid therapy, diabetes mellitus, tuberculosis, pregnancy, indwelling urinary catheter, HIV & neonates who are with Pre-term & Low birth weight [Table 3].

Table 3: Distribution of risk factors associated with candidiasis.

Risk Factors	Number of Samples (n = 100)
Prolonged Antibiotic Therapy	18
Prolonged Hospital Stay	16
Diabetes Mellitus	16
Corticosteroid Therapy	14
Pregnancy	12
Tuberculosis	8
COPD	6
Urinary Catheter	6
Preterm Low Birth Weight	2
HIV	2
Total	100

The distribution of number of different *Candida* species in these 100 isolates are given in Table 4. Accordingly, the species isolated were *C. albicans* (47), *C. tropicalis* (26), *C. glabrata* (12), *C. krusei* (6), *C.parapsilosis* (5), *C.dubliniensis* (3), *C. guilliermondii* (1).

Table 4: Distribution of *Candida* spp and Non-*albicans Candida* (NAC) spp

Species of *Candida*	No. of isolates
Candida albicans	47
Candida tropicalis	26
Candida krusei	6
Candida glabrata	12
Candida parapsilosis	5
Candida dubliniensis	3
Candida guilliermondii	1
Total	100

In the present study Non-*albicans candida* species (53) were isolated at higher rate compared to *Candida albicans* species (47). The species Distribution of different of *Candida* among various clinical specimens is as shown in [Table 5].

Table 5: Distribution of different species of *Candida* among various clinical specimens.

Species of *Candida*	Urine (38)	Sputum (21)	Pus (13)	High Vaginal Swab (10)	Oropharyngeal Swab (8)	Blood (7)	Endotracheal Aspirate (2)	Nail (1)
Candida albicans (47)	18	10	6	6	5	2	0	0
Candida tropicalis (26)	8	9	3	3	2	1	0	0
Candida krusei (6)	2	0	1	0	0	1	2	0
Candida glabrata (12)	6	1	3	0	0	2	0	0
Candida parapsilosis (5)	2	1	0	1	1	0	0	0
Candida dubliniensis (3)	2	0	0	0	0	1	0	0
Candida guilliermondii (1)	0	0	0	0	0	0	0	1
Total	38	21	13	10	8	7	2	1

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Among all the anti-fungal agents, Sensitivity to Amphotericin B was 100% followed by Voriconazole(84%), Itraconazole (69%), ketoconazole(47%) and least sensitivity was shown by fluconazole (45%).Resistance rate of *Candida albicans* to fluconazole, ketoconazole, itraconazole and voriconazole was 46.8%, 63.8%, 25.5% and 19.1% respectively. Amphotericin B showed 100% sensitivity. Resistance rate of non-*albicans candida* species to fluconazole, ketoconazole, itraconazole and voriconazole was 62.26%, 43.3%, 35.8% and 13.2% respectively. *Candida krusei* and *Candida glabrata* were resistant to all the azole group of antifungals and 100% sensitive to Amphotericin B. The sensitivity of *Candida guilliermondii* to fluconazole was susceptible dose dependent and sensitive to all other antifungal agents. [Table 6].

Table 6: Antifungal susceptibility pattern of *Candida* species. S: Sensitivity, SDD: Susceptible dose dependent, R: Resistant

Species of *Candida*	Fluconazole			Voriconazole			Itraconazole			Ketoconazole			Amphotericin - B		
	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R	S	SDD	R
Candida albicans (47)	20	5	22	38		9	35		12	17		30	47		
Candida tropicalis (26)	15		11	24		2	16		10	18		8	26		
Candida krusei (6)			6	4		2			6			6	6		
Candida glabrata (12)			12	10		2	10		2	8		4	12		
Candida parapsilosis (5)	2		3	4		1	4		1			5	5		
Candida dubliniensis (3)	2		1	3			3			3			3		
Candida guilliermondii (1)		1		1			1			1			1		
Total(100)	39	6	55	84	0	16	69	0	31	47	0	53	100	0	0

DISCUSSION

Fungal infections, particularly those attributed to *Candida* species, are frequent complications for hospitalized patients contributing to increased morbidity, mortality and healthcare cost. Furthermore there is increasing prevalence of infections caused by non-*albicans Candida* worldwide with various degree of susceptibility to routinely use antifungal agents indicating the importance of laboratory diagnoses [19]. In this study, it was observed that candidiasis can occur at all ages and in both sexes. Out of 100 patient, majority of the patients belong to age group of 21- 60 years(25) followed by ≥60 years (23) age group. Similar results were seen in study by N Pahwa et al., where out of 237 patients majority of them belonged to ≥60 years (54 out of 237) followed by 19-60 years (38 out of 237) age group[2]. In the study by C Urvashi et al., also it was observed maximum patients with candidiasis belonged to age group ≥70 years (27 out of 100). [3]

In the present study it was observed that out of 100 isolated candidiasis samples, 52% were from females and 48% were from males. Similar results were seen in study conducted by J Gupta et al., where 52.5% were from females and 47.4% were from males,[11] and in study by G Neeta et al., it was observed 55.3% were from females and 44.6% were from males[1]. Also in a study conducted by C Uravashi et al., it was observed again female patients (57%) outnumbered males (43%). [3]

Majority of the isolates in this study were obtained from urine (38%) followed by sputum (21%). khadka et al., also observed in their study that majority isolates were from urine (48%) followed by sputum (42%) samples [18]. However N Kirti et al., study showed that predominant isolates were from sputum (42%) and urine (12%) [16]. This might be due to the fact that the presence of fungi (both yeasts and molds) in sputum has been of increasing interest since the advent of antibiotics and steroids as common therapeutic agents. Moreover, *Candida spp*. is reported as seventh most common nosocomial pathogen in hospital settings causing 25% of all Urinary Tract Infections (UTI) in some of the previous studies. [19-21]

In the present study it was observed that prolonged antibiotic therapy was the most common predisposing risk factor accounting for 18% followed by prolonged hospital stay (16%), Diabetes mellitus (16%) and corticosteroid therapy (14%). In a study by C Urvashi et al., also it was observed that prolonged antibiotic therapy was the most common predisposing risk factor accounting for 26% followed by diabetes (21%) and HIV (16%).[3] Chakrabarthi A and Shivaprakash MR observed higher rate of *Candida* infections in those patients with antibiotics administration of more than seven days and receiving three or more antibiotics.[22] Administration of broad spectrum antibiotics suppresses the endogenous micro flora, permitting fungal overgrowth and any impairment of mucosal immunity is a potential threat for dissemination of *Candida.* Similarly, Kandhari KC and Rama KM found higher occurrence of candidiasis in those individuals with diabetes and HIV.[23]

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In the present study *Candida albicans*(47%) was the most common species isolated followed by *Candida tropicalis* (26%), *Candida glabrata* (12%), *Candida krusei* (6%), *Candida parapsilosis* (5%), *Candida dubliniensis* (3%), *Candida guilliermondii* (1%). The Non-*albicans Candida* species (53%) outnumbered the *Candida albicans* species (47%). Other studies also showed similar results. [1,2,3,4,11,16,18] [Table 7].

Table 7: Comparison of species distribution of *Candida* among different studies.

Author, place, year/*Candida spp*.	***C.albicans* (%)**	***C.tropicalis* (%)**	***C.krusei* (%)**	***C.glabrata* (%)**	***C.parapsilosis* (%)**	***C.dubliniensis* (%)**	**C.*guilliermondii* (%)**	**Others (%)**
S Mondal et al,2013,Nepal	42.5%	27.7%	4.6%	17.5%	7.4%	-	-	-
N Pahwa et al,2014, Indore	42.2%	22.4%	3.4%	3.8%	6.3%	-	0.8%	22.1%
J Gupta et al,Bhopal, 2016	37.17%	46.7%	1.28%	-	9.6%	1.28%	1.92%	1.92%
S Khadka et al, Nepal, 2017	56%	20%	10%	14%	-	-	-	-
G Neeta et al, Chattisgarh, 2019	47.6%	26.2%	8.7%	1.9%	-	-	-	15.6%
N Kriti et al, New Delhi,2021	42%	30%	6%	12%	4%	-	-	-
C Uravashi et al, Manipur, 2022	44%	32%	5%	4%	4%	5%	6%	-

In the present study, disk diffusion method for antifungal susceptibility testing of *Candida* isolates was used. Among the azoles, voriconazole showed the maximum sensitivity of 84%, was the most sensitive and least in Fluconazole (45%). Amphotericin B showed 100% sensitivity to all the isolates of candida spp. The sensitivity rate of Itraconazole was 69% and ketoconazole was 47%. Isolates of *Candida krusei* and *Candida glabrata* species were resistant to all the azoles. [1,4,3,11,16,18] [Table 8].

Table 8: Comparison of Anti-fungal susceptibility pattern of *Candida* species among different studies S: Sensitivity, SDD: Susceptible dose dependent, R: Resistant

***Candida spp*. with antifungal/Authors with places and years of publication**		**S Mondal et al, 2013, Nepal (%)**	**J Gupta et al,Bhopal, 2016**	**S Khadka et al, Nepal, 2017(%)**	**G Neeta et al, Chattisgarh, 2019(%)**	**N Kriti et al,New Delhi,2021**	**C Uravashi et al, Manipur, 2022(%)**
Candida albicans	F	89.4	86.2	82.3	89.8	85	79.6
	V	-	100	-	100	85	84.1
	It	-	89.65	-	-	-	79.6
	K	92	-	10.8	-	-	63.6
	Amp	99.9	100	-	97.9	100	93.2
Candida tropicalis	F	80.6	83.5	80	89.8	80	31.3
	V	-	98.6	-	100	86	81.3
	It	-	86.3	-	-	-	56.3
	K	84.4	-	20	-	-	50
	Amp	99.9	100	-	97.9	100	65.6
Candida krusei	F	0	0	80	89.8	0	0
	V	-	100	-	100	0	100
	It	-	0	-	-	-	40
	K	80	-	20	-	-	40
	Amp	100	100	-	100	100	60
Candida parapsilosis	F	100	80	-	-	-	50
	V	-	100	-	-	-	100
	It	-	86.6	-	-	-	25
	K	100	-	-	-	-	0
	Amp	100	100	-	-	-	50

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CONCLUSION

In the present study it is seen that there is a changing trend of rise in the incidence of non *albicans Candida* over *Candida albicans*. The increase in predisposing factors in the recent years has led to increasing incidence of *Candida* infections. Thus, early speciation of *Candida* isolates is helpful to restrict the empirical use of antifungal agent and also greatly influence the treatment options available for the clinicians and hence will be beneficial for the patients as some *Candida* species are intrinsically resistant to few antifungal. Extensive study is required for the Species level identification of *Candida* and their antifungal sensitivity testing should be performed to achieve better clinical outcome. This may in turn help to develop guidelines on empiric therapy for invasive fungal infections.

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