

## A six-year hospital-based surveillance study on burden of esophageal candidiasis in Gangtok, Sikkim

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### ABSTRACT

**Background and Objectives:** Esophageal candidiasis once thought to be restricted amongst immunocompromised patients is being increasingly reported among non-immunocompromised individuals. It is debilitating and if not treated well may cause chronic long-lasting infections. The objective of this study was to identify the various species of *Candida* causing esophageal candidiasis and analyse their antifungal susceptibility pattern.

**Materials and Methods:** This was an observational, prospective study. Total of 108 patients who attended the Gastroenterology Department of Sir Thutob Namgyal Memorial Hospital, Govt of Sikkim, Gangtok, India between July 2012 – May 2018 were included in the study. They had complaints of upper gastrointestinal disturbances and chronic dyspeptic symptoms that required an endoscopy. Esophageal biopsy and brushings were taken and were transported to Microbiology Department. They were subjected to microscopic observation, fungal culture on Sabourauds dextrose agar. Preliminary species identification was done by chlamyospore formation and growth characteristics on CHROMagar *Candida*. Species confirmation and antifungal susceptibility testing was done on VITEK 2 system at Microbiology Department, Kasturba Medical College and Hospital, MAHE, Manipal, Karnataka, India.

**Results:** A total of 108 patients were screened among which 73 samples were positive for *Candida* species and species identification and antifungal susceptibility was performed. Forty five isolates were found to be *C. albicans*, 8 were *C. glabrata*, 4 were *C. tropicalis*, 3 were *C. lusitanae* 2 were *C. krusei*, 2 were *C. lipolytica* and 1 was *C. parapsilosis*. Eight isolates could not be identified and were recorded as *Candida* spp. *C. albicans* isolates were predominantly sensitive strain with susceptibility of 95% for both amphotericin B and fluconazole and 100% for caspofungin. *C. glabrata* showed high resistance to fluconazole with one isolate showing intermediate resistance to caspofungin.

**Conclusion:** Upper gastrointestinal symptoms even in non-immunocompromised patients need to be screened by endoscopy to rule out esophageal candidiasis. With the emergence of drug resistant non albicans *Candida* species diagnostic testing laboratories should include *Candida* species identification and antifungal susceptibility testing facility to provide effective patient care.

**Keywords:** *Candida*; Candidiasis; Esophagitis; Antifungal; Endoscopy; Amphotericin B; Fluconazole; Caspofungin

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## INTRODUCTION

The spectrum of Candidiasis encompasses wide range of infections ranging from mucocutaneous (oral, oesophageal and vulvovaginal) to systemic involvement in the host (1). Incidence of esophageal candidiasis (EC) in general population is between 0.32% to 5.2% (2) and it is one of the most common opportunistic infections in immunocompromised patients (1) seen in 9.8% of human immunodeficiency virus (HIV) infected patients (3). They are increasingly being diagnosed in non-immunocompromised individuals as seen in 0.32% in Korea (4), 1.6% in Japan (3) and 3.6% in Tanzania (5). In India, it has been reported in 44.2%-61% (6, 7) of HIV infected patients. Diabetes mellitus, use of antibiotics, proton pump inhibitors, steroids, smokers and alcohol consumption are risk factors that predisposes to oesophageal candidiasis (3, 4, 8).

Over the decades, the species distribution spectra for *Candida* species causing human infections have changed. *Candida albicans*, previously the dominating pathogen, accounts for only half the isolates (9). Non-*albicans Candida* species have emerged as a formidable pathogen, many of which are highly resistant to the currently available antifungals (10). More often therapeutic failure is encountered in the treatment of non-*albicans Candida*.

Unlike the various classes of antibiotics available for bacterial infections there is paucity of antifungals available for use, namely azoles, echinocandins and polyenes. High rates of fluconazole resistance in non-*albicans Candida* and an increase in acquired resistance to caspofungin is a matter of concern and poses a threat to human health (10).

The present hospital-based study was carried out to estimate the burden of EC and to study their antifungal susceptibility along with associated risk factors in Sikkim provinces.

## MATERIALS AND METHODS

The study was executed at Department of Microbiology and Gastroenterology, Sir Thutob Namgyal Memorial Hospital, Gangtok and Department of Microbiology, Kasturba Medical College (KMC), Manipal. It was a prospective, cross-sectional, observational study. A prior clearance from the Institutional Ethics Committee was obtained.

**Inclusion criteria.** One hundred and eight patients attending Gastroenterology Department of Sir Thutob Namgyal Memorial Hospital, Gangtok between July 2012 – May 2018 with complaints of upper gastrointestinal disturbance – epigastric / retrosternal pain and with chronic dyspeptic symptoms requiring an endoscopy were included in the study. All patients were screened for HBsAg, HCV and HIV.

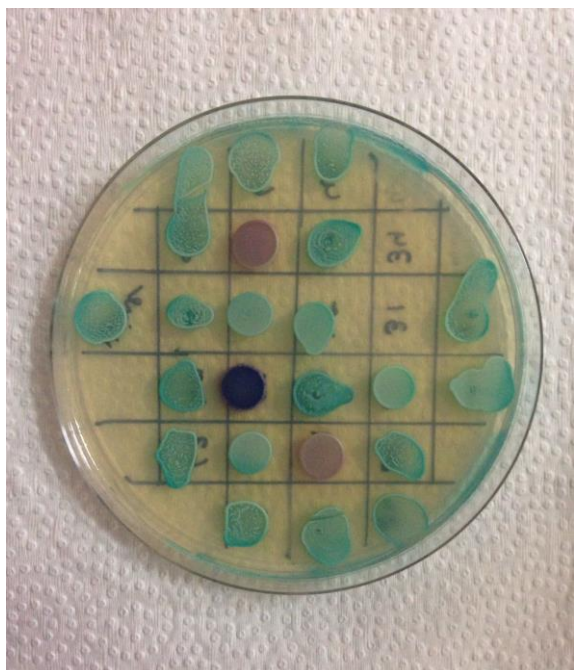
Upper Gastrointestinal (GI) endoscopy was performed using flexible endoscope (Olympus Elvis Exera 190). Curdy white to yellowish precipitates resistant to water syringing/washing were removed and biopsy sample / brushings were taken.

**Preliminary testing.** Biopsy samples were sent to the microbiology laboratory in sterile universal containers with normal saline. Esophageal brushings were directly inoculated on Sabourauds Dextrose Agar (HiMedia Labs Pvt. Ltd.) with gentamicin (0.5 mg in 100 ml) in duplicate and onto clean glass slides for bright field microscopy observation. They were transported to Microbiology Lab where preliminary Gram staining was done and yeast like growth was further tested for Germ tube and chlamydiospore formation. The presumptive identification of isolated organism by conventional methods were noted, and the isolates were stored at -20°C.

**Screening on chromogenic media.** All *Candida* isolates were transported to Department of Microbiology, Kasturba Medical College, Manipal, Karnataka where screening by growth on CHROMagar *Candida* for typical colony characteristics and were identified depending on their color and pattern of growth by following manufacturers instruction (Fig. 1).

**Automated identification and antifungal susceptibility testing.** The isolates were then tested on automated Vitek 2 system (bioMérieux, France). Vitek 2 system uses advanced calorimetric method to identify routine clinical isolates of *Candida*. Advanced expert system software provides accurate fingerprint recognition of antifungal resistance which analyses MIC patterns. Isolates were then tested against the following antifungals -amphotericin B (AMB), fluconazole (FLC), caspofungin (CAS), flucytosine (5FC), micafungin (MFG), voriconazole (VRC) and itraconazole (ITC).

**Statistical analysis.** Descriptive analysis, Chi

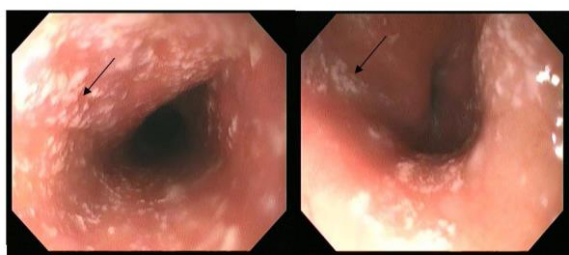


**Fig. 1.** CHROMagar *Candida* - green apple colonies of *C. albicans*, light pink colonies of *C. glabrata* and steel blue colonies of *C. tropicalis*

square test and fisher's exact test were used for analysing the data for risk factors. A p value < 0.05 was considered to be significant. Descriptive analysis was done using Microsoft excel 2010 and inferential analysis was done using SPSS version 2019.

## RESULTS

**Patient demographics.** A total of 3,329 Upper GI endoscopy was performed between 2012 and 2018 among which, 108 patients with endoscopic findings of EC (Fig. 2) were screened for HIV, HBsAg and HCV and only one patient was found to be positive for hepatitis B while the rest were negative for both



**Fig. 2.** Endoscopic picture showing white plaques (arrow head) which were sent for culture

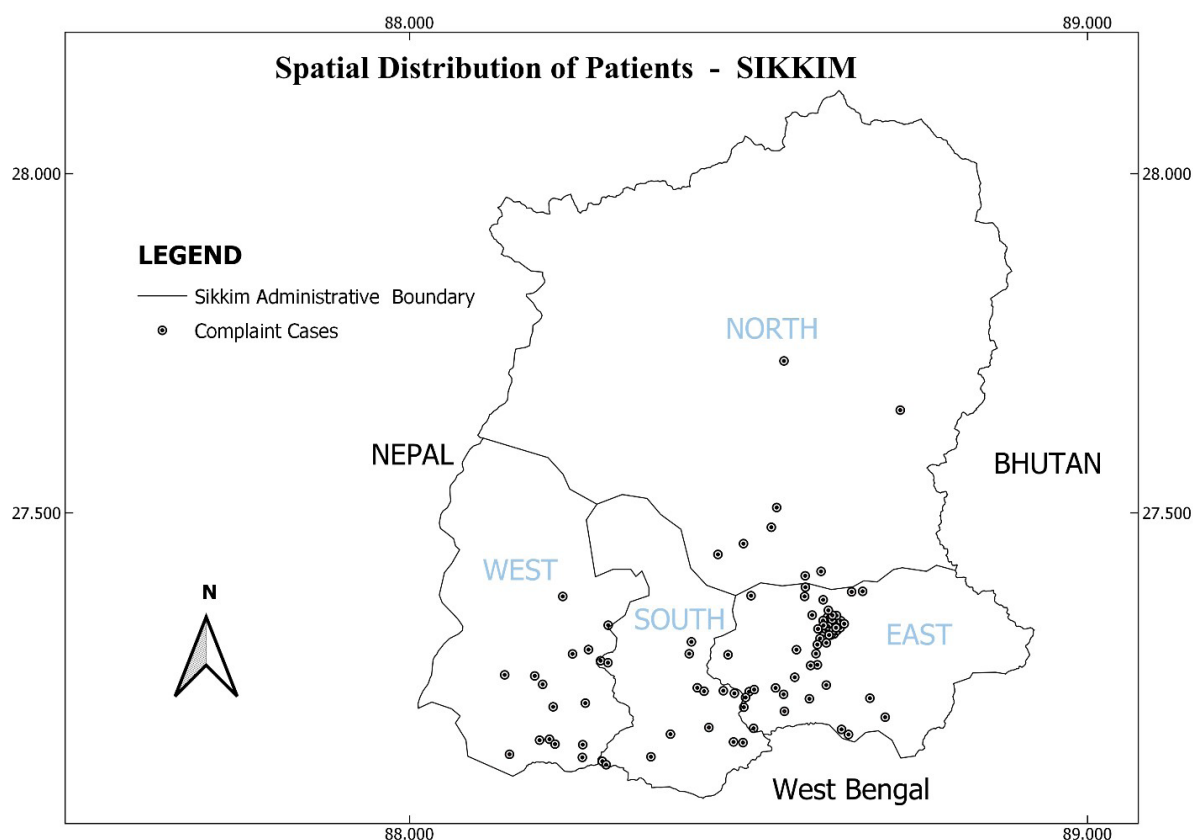
HIV, hep B and hep C.

Among the 73 patients with laboratory confirmed EC, 44 were male and 29 were females. The mean age was  $50.59 \pm 18.61$  years with the maximum number of patients (23.2%) in the 51-60 years age group. Cases were observed in all three ethnic community – Nepali (72.6%), Bhutia (17.8%) and Lepcha (9.58%) - of Sikkim with higher incidence among the Nepali community. EC patients came from all the four districts of the state- North (8.21%), South (21.9%), East (56.16%) and West District (13.69%) with maximum number from East district (Fig. 3).

**Clinical features and risk factors.** The most common presenting symptom was epigastric pain (53.42%). Classical presentation of dysphagia was seen in (12.32%) patients, along with vomiting (12.32%) (Table. 1). Among the risk factors assessed, alcohol consumption was seen in (49.31%), smoking (28.76%), chewing of pan/gutka in (35.61%), history of cancer in the family (2.73%) and treatment with steroids in (1.36%).

**Screening and culture.** All samples were processed by Grams Stain, 10% KOH microscopy, culture on SDA and chlamyospore formation on Cornmeal agar. *Candida* was demonstrated microscopically on Grams Stain and KOH in all 108 patients. 73 samples were positive in culture but only 71 samples could be sent for further confirmation as two isolates could not be revived after being stored at -20 for 2 to 3 months in SDA before they were shipped to partner institute for further confirmation.

**Automated identification.** Seventy one samples were sent to Department of Microbiology, Kasturba Medical College and Hospital, Manipal, Karnataka for species identification on CHROMagar *Candida*, VITEK 2 and antifungal susceptibility to be performed by VITEK 2. Among the 71 isolates, eight were not read correctly by Vitek 2. As molecular testing was not included in the study protocol the unknown isolates were not tested further by other methods for species identification. Out of the 63 isolates identified by VITEK 2, 53 were found to be *C. albicans*, 8 were *C. glabrata* (currently *Nakaseomyces glabrata*), 4 were *C. tropicalis*, 3 were *C. lusitaniae* (currently *Clavispora lusitaniae*), 2 were *C. krusei* (currently *Pichia kudriavzevii*), and 1 was *C. parapsilosis* (Fig. 4).



**Fig. 3.** Epidemiological map showing the distribution of patients with longitude and latitude – Map created with open source software called Quantum GIS.

**Antifungal susceptibility.** Antifungal susceptibility results are shown in Table 2. *C. albicans* isolates were predominantly sensitive strain with susceptibility of 95% for both amphotericin B and fluconazole and 100% for caspofungin. *C. glabrata* showed high resistance to fluconazole with one isolate showing intermediate resistance to caspofungin. Multi Drug Resistant (MDR) strains of *C. krusei*, *C. lusitanae* were encountered. *C. tropicalis* and *C. parapsilosis* were sensitive strains against fluconazole, voriconazole, itraconazole and caspofungin.

Association between alcohol consumption and smoking with development of esophageal candidiasis was not significant P-value-0.397 and 0.276 respectively.

## DISCUSSION

Isolation of *Candida* species from an individual's sample does not always signify an underlying fungal infection (11). The oral basal mycobiome of healthy individuals comprises predominantly of *Candida*

species (12). The immune system along with interaction with bacterial microbiome and the intact epithelial lining limits *Candida* from infecting the host (13). Colonisation followed by invasion leads to EC often due to decreased host immunity (14).

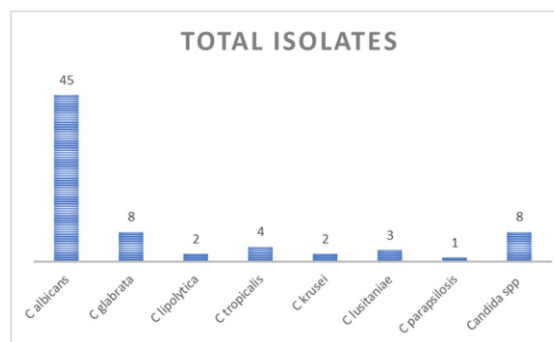
EC among patients with HIV has declined from 51.8% to 16.7% (15). EC has been increasingly reported in non-HIV patients (3, 15) with rise in EC prevalence from 0.6% to 2.5% between 2003- 2014 (3). Prevalence of EC among non immunocompromised patients in our study was 2.19% (73/3, 329) which is higher than in other studies done in Pakistan 0.34% (16), Korea 0.32% (4), Japan 1.5% (3) but lower than Tanzania 3.6% (5). The efficacy of the Highly Active Anti Retroviral Therapy (HAART) (17) introduced in Sikkim under the National Aids Control Organization which prevents development of immunodeficiency and opportunistic infections (18) may be the reason for absence of HIV infected patients with EC in our study.

Pathogenicity in healthy individuals has not been well elucidated apart from its association with risk

**Table 1.** Associated symptoms, risk factors and demographic profile of patients.

Common symptoms	Number
Epigastric pain	39
Dysphagia	09
Vomiting	09
Jaundice	14
Cirrhosis of Liver (ALD)	11
Breathlessness	03
Loss of Appetite	08
Malena	03
AGE	08
Bacillary Dysentery	01
Diarrhea	01
Anemia	02
Ascitis	02
Hematemesis	05
COPD	01
Fever	05
Anxiety Disorder	01
Risk Factors	Number
Alcohol Consumption	36
Smoker	21
Chews pan/ Gutka	26
Diabetes Mellitus	
Liver Cirrhosis	11
Cancer	02
Treatment with steroids	01
Gender	Number
Male	44
Female	29
Ethnic Community	Number
Nepali	53
Bhutia	13
Lepcha	13
Occupation	Number
Agriculturist	20
Service (Govt/Pvt)	19
Skilled worker	07
Business	08
Housewife	24
Student	04
Age group	Number
11-20	03
21-30	08
31-40	14
41-50	11
51-60	17
61-70	07
71-80	08
81-90	05

ALD- Alcoholic Liver Disease. AGE- Acute Gastroenteritis.  
COPD- Chronic Obstructive Pulmonary Disease.

**Fig. 4.** Total *Candida* isolates. The numbers over the graph depict numbers isolated.

factors that may make an individual more prone to EC (2-5, 16). Although non immunocompromised, most of the patients with EC were associated with various risk factors like alcohol consumption (49.31%), smoking (28.76%) and chewing tobacco in the form of Pan and Gutka (35.61%) but there was no correlation of alcohol consumption or smoking with EC in our study. Conflicting literature reviews are available for alcohol consumption as a risk factor. Choi et al. has shown a significant correlation with  $p < 0.001$  (4), similar findings were seen in Tanzania with an OR of 17.1, 95% CI for alcohol use and OR of 8.3, 95% CI for smoking (5). In another study by Alsomelli et al. smoking was more often seen in EC while alcohol consumption was found to be protective (14). In Contrary, Takahashi et al. found no correlation between alcohol consumption and EC while smoking had marginal association (3). Alcohol and smoking both weaken the local immunity of the esophageal epithelium and facilitate colonization of esophagus by pathogens from the oral cavity with invasion and proliferation by *Candida albicans* (2, 16).

The most common age group was 51-60 years (23.28%) with the mean age of  $50.59 \pm 18.61$ . Similar findings have been reported by previous investigators where the mean age was  $53.1 \pm 14.1$  years (2),  $60.3 \pm 15.0$  (3),  $52.9 \pm 14.6$  (16) and the median age worldwide being 55.5 (15). Increasing age has been recognised as a risk factor for EC in non-HIV-infected patients as this age group has an impaired immunity due to defects in the hematopoietic bone marrow and in peripheral lymphocyte migration, maturation, and function (8). Another reason may be due to the reduction in cellular immunity of the epithelial layer in patients with increased age thus leading to colonization by *Candida* (2, 3).

*C. albicans* was the most frequently isolated organism from patients with EC as was observed by

**Table 2.** Antifungal susceptibility test results

SPECIES	AMPHOTERICIN B			FLUCONAZOLE			VORICONAZOLE			ITRACONAZOLE			CASPOFUNGIN			FLUCYTOSINE			MICA FUNGIN					
	(n = 46)			(n= 46)			(n= 46)			(n= 11)			(n= 4)			(n= 34)			(n= 24)					
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
<i>C. albicans</i>	95.6	2.1	2.1	95	-	4.3	100	-	-	100	-	-	100	-	-	94	(2.9)	(2.9)	100	-	-			
	(44)	(1)	(n= 1)	(44)		(2)	(46)			(11)			(4)			(32)	(1)	(1)	(24)					
<i>C. glabrata</i>		8	I		(n= 7)			(n= 8)			(n= 7)			(n= 8)			(n= 1)			(n= 1)				
	S		R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Sensitivity (%)	100	-	-	14	85		100	-	-	100	-	-	87	12.5	-	100	-	-	100	-	-			
	(8)			(1)	-	(6)	(8)			(7)			(7)	(1)		(1)			(1)					
<i>C. lusitaniae</i>		(n= 3)			(n= 1)			(n= 3)			Not tested			(n= 1)			(n= 3)			(n= 1)				
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Sensitivity (%)	100	-	-	-	-	100	100	-	-	-	-	-	-	-	100	100	-	-	-	-	100	-	-	100
	(3)					(1)	(3)								(1)	(3)								(1)
<i>C. tropicalis</i>		(n= 3)			(n= 3)			(n= 3)			(n= 2)			(n= 3)			Not tested			(n= 1)				
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Sensitivity (%)	66	33.3	-	100	-	-	100	-	-	100	-	-	100	-	-	-	-	-	-	-	-	100	-	-
	(2)	(1)		(3)			(3)			(2)			(3)									(1)		
<i>C. krusei</i>		(n= 2)			(n= 2)			(n= 2)			Not tested			(n= 2)			(n= 2)			(n= 2)			(n= 2)	
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Sensitivity (%)	100	-	-	-	-	100	100	-	-	-	-	-	50	50	-	-	-	100	100	-	-			
	(2)					(2)	(2)						(1)	(1)				(2)	(2)					
<i>C. parapsilosis</i>		(n= 1)			(n= 1)			(n= 1)			(n= 1)			(n= 1)			(n= 1)			Not tested				
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Sensitivity (%)	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-	-	-	-			
	(1)			(1)			(1)			(1)			(1)			(1)								

n - number of isolates tested , S- Sensitive, I- Intermediate, R-Resistant.

Maninder et al. 70.49% (7) and Klieman et al. 96.2% (8). Our findings were also similar where 61.64% of the isolates were *C. albicans*. EC requires colonization and invasion by *Candida* and as *C. albicans* is the most common yeast colonizing the oral cavity and the gastrointestinal tract of humans (13).

Species of *Candida* causing infections has changed over the last decade with a shift towards Non-*albicans Candida*- *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. parapsilosis* (9, 19, 20). Pahwa et al. have observed in a tertiary care hospital in central India a predominance of non-*albicans Candida*- 57.8% over *C. albicans* (21). *C. glabrata* is a common pathogenic candida infecting human (9) and has a prevalence of

1.6% to 3.8% (8) in esophageal samples. In our study we isolated 10.95% of *C. glabrata*. *C. tropicalis* was the most common non-*albicans Candida* isolated in studies conducted in other Indian states (20, 21) but we encountered only 5.47% of *C. tropicalis*. Geographical difference between the places where the studies were conducted – tropical climate vs temperate Himalayan region may be responsible for this difference. Similar perspective was discussed where global trends in the distribution of *Candida* species were reviewed (1, 22).

Widespread use of antifungal agents has shown an increase in drug resistant strains of *Candida* species which were once susceptible to all agents (10).

Susceptibility profile to different antifungal agents varies according to the *Candida* species (23). We encountered 4.3% of *C. albicans* resistant to fluconazole (FLC), 2.1% to amphotericin B (AMB) and 2.9% to flucytosine (F5C). No cross resistance was seen for other azole groups nor the echinocandins. This is in concordance with the global resistance rates of *C. albicans* (0-7.8%) for FLC, and Indian study of 4.65% (7) and 5.9% (22) which is considerably less when compared to resistance rates for *C. glabrata* and *C. tropicalis*, 0-76% and 0-66.6% respectively (10). In the United States *C. albicans* has FLC resistance of 0.5-2% and *C. glabrata* has high rates at 11-13% (10).

FLC resistance demonstrated by the non-*albicans Candida* in our study was 64.2% which is relatively high when compared to other Indian study 22.2% (7), 9.5% (20). 85% of the non-*albicans Candida* resistant to FLC were *C. glabrata* strains. FLC is a fungistatic agent and acquired resistance develops easily in its presence (23). Increased FLC resistance has been reported in *C. tropicalis*, one of the most commonly isolated non-*albicans Candida* (22, 23). It is interesting to note that the *C. tropicalis* isolated in this study were sensitive to all the antifungals tested, except for one strain which showed intermediate resistance to AMB.

This investigation suggests EC is present in Sikkim although the prevalence is low when compared to other infectious disease. The possibilities of EC in patients should be considered even in the absence of predisposing factors such as HIV infection or an immune compromised condition.

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