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RESEARCH ARTICLE

Oral Candida colonization and infection in cancer patients and their antifungal susceptibility in a tertiary care hospital.

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Oral candidiasis is a common fungal infection affecting cancer patients. Although most cases are due to *C. albicans*, non-albicans strains have increasingly been implicated in causing this disease. The aim of this study was to investigate the epidemiology of oral yeast colonization and infection amongst cancer patients and their antifungal susceptibility. A total of 150 cancer patients and 150 healthy controls were included in the study. Cases included patients with solid, head and neck or hematological malignancy. Oral examination was done and oral swabs taken from all the participants for yeast culture, identification and susceptibility testing to fluconazole and voriconazole. Amongst the cancer group, 75 (50%) had solid organ malignancy, 45 (30%) had hematological malignancy and 30 (20%) had head & neck malignancy. Total colonization was prevalent in 50% and oral candidiasis in 30% of all cancer patients. Highest rate of total colonization and candidiasis was seen in head and neck cancer patients (77% and 63% respectively) and in patients receiving chemotherapy and radiotherapy together (72.97% and 56.75% respectively). Age ≥ 60 years and recent oral fungal infection were associated with Candida carriage. *C. albicans* was the most common species (74.39%) causing colonization and candidiasis with 100% susceptibility to the two azoles. Overall sensitivity of Candida spp. to fluconazole and voriconazole was 92.68% and 100% respectively.

Introduction

Candida organisms that exist predominantly in a unicellular form are small (4-6µm), thin-walled, ovoid cells that reproduce by budding. Although there exist more than 150 species, only a small percentage are frequently implicated as human pathogens, notable being *C. albicans*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. kefyr*, *C. lusitaniae*, *C. dubliniensis*, and *C. glabrata*. [1]

Oral candidiasis is a common fungal infection affecting cancer patients. Over the years an incidence of 7.2–52% has been reported depending on treatment interventions and the type and stage of the malignancy. [2-6] Majority of oral infections are due to *Candida albicans* but non-albicans strains such as *C. glabrata* and *C. tropicalis* have increasingly been implicated in causing disease. [7, 8] Patients often progress from asymptomatic yeast carriage to oral candidiasis presenting with white pseudomembraneous plaques (thrush) or erythematous

ulcerations. Cell-mediated host immunity plays an important role in the control of fungal infections and cytotoxic chemotherapy, radiation or malignancies are known to compromise it, predisposing a person to infections due to fungi. [9] Oral candidiasis contributes considerably to morbidity when it presents with pain or burning leading to subsequent poor nutrition or even invasive infections such as esophagitis or candidemia. [10, 11] Other risk factors that promote development of this oral condition include diabetes, use of broad spectrum antibiotics, corticosteroids, AIDS, and organ transplantation. [12-14]

Studies from around the world suggest that there is a difference in the incidence of oral yeast colonization and infection amongst different cancer groups, with majority of them having focused on single cancer populations, such as head and neck cancer. Very few studies have compared the epidemiology of oral candidiasis between different cancer groups and types. Also some centers have reported an increase in resistance to first line anti-fungal agents such as fluconazole in Candida spp. due to the widespread use of these agents as prophylaxis in neutropenic cancer patients. Early detection and identification of fungal pathogens for targeted antifungal therapy is of paramount importance.

The study was thus carried out at Sher-i-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, Kashmir, a 690 bedded tertiary care centre in Northern India to compare the epidemiology of oral candidiasis (colonization and infection) in different cancer groups and types, to study the risk factors associated with acquisition of oral candidiasis in cancer patients and to get an insight into the susceptibility profile of the various species of Candida isolated.

Material and Methods

This prospective study was performed in the Department of Microbiology in collaboration with Departments of Medical Oncology, Clinical Hematology and Radiotherapy at SKIMS, Srinagar,J&K. The study was approved by Institute's ethical committee (SIMS131/IEC-SKIMS/2012-4905-06)

Patients with solid tumors, head and neck cancer or hematological malignancy coming to the daycare centre or admitted to the wards for chemotherapy/radiotherapy from January 2012 onwards were included in the study. Samples from healthy persons were also taken, which served as controls. An informed consent was taken from both cases and controls (selected randomly) prior to their enrolment into the study. Age, sex, use of antibiotics, any chronic ailment, type of cancer, surgical procedure in the preceding two months, recent history of oral fungal infections, treatment taken for that and use of dentures were noted for all the participants. [15, 16]Patients who had taken antifungals in the past four weeks were not included in the study. And those already recruited were not included subsequently.

A total of 150 samples from cases and 150 from healthy controls were included in the study. An examination of the oral cavity of the patients was performed and a sample from tongue, buccal mucosa and labial sulcus was taken with a sterile pre-moistened swab. [17]Ten samples (5 from patients of solid malignancies, 3 from patients of hematological malignancies and 2 from patients of head-neck cancer) were collected per week. An equal number of samples were taken from healthy attendants of the patients (controls). All samples were processed for yeast isolation in Microbiology laboratory on the same day.

Oral Candida carriage or total colonization (colonization+ candidiasis) was defined as presence of yeasts in the oral cavity irrespective of signs and symptoms. Oral candidiasis was defined as presence of Candida spp. in the oral cavity together with signs and symptoms of oral candidiasis like inflammation/mucositis and/or presence of white plaques confirmed microbiologically by the presence of yeasts and/or hyphae or pseudohyphae on potassium hydroxide–treated smears of oral swabs. [17, 18]

For all patients, the clinical diagnosis was confirmed microbiologically by the presence of yeasts and hyphae or pseudohyphae on 10% potassium hydroxide (KOH) preparation of oral swabs. [18] Oral swabs were directly inoculated on Hichrome Candida differential agar on the day of collection and incubated at 37°C for 24 to 48 hours till colonies became visible on the medium. [15,19, 20]Preliminary speciation of the yeast isolates was done on the basis of colour of colonies on Hichrome agar, germ tube formation and microscopic morphology on corn meal agar (CMA) (Fig-1). Confirmation of the species was done by assimilation of sugars viz; glucose,

sucrose, maltose, lactose, cellobiose, mellibiose, trehalose, raffinose, xylose, galactose, dulcitol. [21, 22] In addition rhamnose assimilation and sucrose, maltose and trehalose fermentation was also done for some isolates. [23]

Antifungal susceptibility testing of Candida spp.to fluconazole (25µg) and voriconazole (1µg) was performed by disk diffusion method in accordance with the CLSI M44-A guidelines. [24,25,26,27,28,29] Briefly, discs containing these antifungal agents were applied to the surface of Mueller Hinton Agar (supplemented with 2% glucose and 5µg/ml methylene blue dye) on which lawn culture of the clinical isolate had been done. Following incubation at 37°C for 24 to 48 hours, plates were examined and zones of inhibition surrounding the discs measured and compared with established zone size ranges for individual antifungal agents.

CLSI guidelines were followed for the interpretative criteria of fluconazole and voriconazole disk diffusion testing. For fluconazole, a zone diameter of 19 mm was taken as susceptible, 15 to 18 mm; susceptible dose dependent and 14 mm as resistant. For voriconazole, a zone diameter of 17 mm was taken as susceptible, 14 to 16 mm; susceptible dose dependent and 13 mm as resistant. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 and *C. albicans* 90028 were used for quality control.

Results

A total of 150 patients with various malignancies, along with 150 healthy age and sex matched controls were included in the study, over a period of 14 months. Recruited patients included 100 males and 50 females, while the control group consisted of 97 males and 53 females. The age of the patients ranged from 1 to 75 years, with a mean age of 42.22years. In cases, a higher prevalence of total colonization and oral candidiasis was seen in males (54% and 33% respectively) as compared to females (42% and 24% respectively). In the control group, total colonization was higher in females (24.53%) compared to males (16.49%) whereas prevalence of oral candidiasis was slightly higher in males (3.09%) as compared to females. The difference in colonization and candidiasis between the two genders was not significant amongst cases or controls.

Amongst the cases (n=150), 75 (50%) had solid organ malignancy, followed by 45 (30%) patients who had a hematological malignancy and 30 (20%) patients who had head & neck malignancy. Total colonization was prevalent in 50% (75) of all cancer patients and 19.33% (29) of the control group, whereas clinical and microbiological evidence of oral candidiasis was seen in 30% (45) of the patients and 2.7 % (4) of the controls. The difference in the prevalence of candidiasis and total colonization in cases as compared to controls was significant ($p<0.0001$).

Total colonization was highest in head & neck cancer patients (77%) followed by patients with hematological malignancies (49%) and least in patients with solid malignancies (40%). Prevalence of oral candidiasis was also found to be higher for head & neck cases (63%); with almost a similar prevalence in cases with hematological malignancies (22%) and solid tumors (21%). The prevalence of total colonization ($p=0.003$) as well as oral candidiasis ($p<0.0001$) was significant for head and neck cancer patients in comparison to those who had other malignancies.

Total colonization as well as oral candidiasis was higher in patients receiving chemotherapy and radiotherapy together (72.97% and 56.75%) as compared to patients receiving chemotherapy alone (42.47% and 21.23%). The difference was significant both for total colonization ($p=0.001$) and candidiasis ($p<0.0001$) for the two groups. Other risk factors like oral antibiotic intake, H/O recent oral fungal infection, use of dentures, age $\geq$ 60 years and chronic ailments like diabetes and surgery in preceding two months were seen to be associated more with candida colonization Table 1. Multivariate analysis by Binary logistic regression (Forward selection) showed that out of these variables age $\geq$ 60 years ($p=0.024$) and H/O recent oral fungal infections ($p=0.010$) were statistically significant for acquisition of colonization. Besides these two variables another variable i.e. treatment (chemotherapy or chemo radiotherapy) also had significant association with colonization ($p=0.008$).

A total of 82 Candida isolates were recovered from 75 cancer patients which included *C. albicans* (61), *C. glabrata* (7), *C. tropicalis* (5), *C. parapsilosis* (4), *C. guilliermondii* (2), *C. lusitaniae* (2) *and C. krusei* (1) and 30 Candida isolates were recovered from 29 controls which included *C. albicans* (17), *C. glabrata* (4), *C. tropicalis* (4), *C. parapsilosis* (2), *C. guilliermondii* (2) and *C. kefyr* (1). Most of the patients were colonized by single Candida spp. (90.67%). *C. albicans* was the most common species causing colonization both in cancer patients (74.39%) as

well as controls (56.67%). It was also the most common cause of candidiasis in both the groups (65.4% in cases and 75% in controls). *C. glabrata* was the second most common species followed by *C. tropicalis* and *C. parapsilosis* to cause colonization as well as candidiasis in cancer patients. Amongst controls, although *C. glabrata* and *C. tropicalis* were the second most common cause of colonization, oral candidiasis was commonly caused by *C tropicalis*.

The antifungal susceptibility of 112 Candida species (82 from cases and 30 from controls) was performed using fluconazole and voriconazole discs. (Fig. 2) Among cases as well as controls all *C. albicans* isolates were sensitive to fluconazole (100%). Among non-albicans species in cancer patients, only *C. glabrata* and *C. krusei* showed resistance to fluconazole i.e. 4 (57.14%) out of 7 *C. glabrata* isolates were resistant to fluconazole and a single isolate of *C. krusei* that was recovered was resistant to fluconazole. Among non-albicans species recovered from controls, all the 4 (100%) isolates of *C. glabrata* were resistant to fluconazole whereas all other non-albicans species were sensitive to it. All the isolates of Candida recovered from cases as well as controls were sensitive to voriconazole. The sensitivity profile of the Candida isolates recovered from cases and controls is shown in Fig. 3&4. The overall sensitivity for fluconazole was 92.68% whereas that for voriconazole was 100%. Fluconazole resistance in non-albicans species was highest in solid malignancies (40%) followed by hematological cases (25%) with least resistance seen in head & neck cases (18.18%).

Discussion

Oropharyngeal candidiasis is a common fungal infection in cancer patient and currently ranks as the most common human fungal disease. [16] Cytotoxic chemotherapy, radiation or malignancy per se in these patients can lead to compromised cell mediated immunity; something that normally keeps fungal infections in check. [15]Little is known about the epidemiology of oral Candida colonization and infection in developing countries. [30]The present study was carried out to compare the epidemiology of oral Candida colonization and candidiasis in different cancer groups in our state and perform antifungal susceptibility of the isolates recovered. The study compares yeast colonization and infection in three major cancer groups namely solid tumors, hematological and head and neck malignancies.

Total colonization was prevalent in 50% of all cancer patients of which oral candidiasis was seen in 30%. Similar results were seen by Schelenz et al. who in their study found an overall colonization rate of 56.8% however a lower rate of candidiasis (18.9%) among cancer patients was seen by them. [15]Al-Abeid et al. on the other hand reported a higher rate of colonization i.e. 72.6% in Jordanian cancer patients. [16]

Total colonization (77%) and oral candidiasis (63%) was highest in head & neck cancer patients owing to the fact that apart from having other risk factors, that predisposed them to colonization and infection by Candida spp. nearly all the patients had received radiotherapy in addition to chemotherapy, in this subset of patients which compounded the problem. Redding et al. found a colonization rate of 73% in head and neck cancer patients, comparable to the results obtained in our study. [19]Although a higher incidence of colonization was seen in solid tumor patients (64.6%) by Schelenz et al., the authors found that patients with head and neck cancer had highest rate of infection (29.2%). [15]

A significantly higher rate of total colonization and oral candidiasis was seen in patients receiving chemotherapy and radiotherapy together as compared to patients receiving chemotherapy alone in this study. It is well known that radiotherapy leads to mucositis, xerostomia and mucosal damage, which promote yeast infection. Also, neutropenia due to prolonged chemotherapy, disruption of mucosal barrier and overall damage to cell mediated immunity increases the risk of infection. [15] Similar trends have been seen in various studies conducted across the world. Amador et al. found that radiotherapy induced hypo salivation encourages oral candida colonization that often leads to oral/pharyngeal candidiasis. [31] Similarly Dahiya et al. found an increased incidence of oropharyngeal candidiasis in patients receiving concomitant radiotherapy and chemotherapy. [20] Jham et al. in their study evaluating the oral health status of 207 head and neck cancer patients before, during and after radiotherapy found the incidence of candidiasis to be as high as 45.8% during radiotherapy. Mucositis was seen in 61.7% of the patients receiving radiotherapy. [3]

Of all the risk factors studied in this study, age $\geq$ 60 years and recent H/O oral fungal infections were found to be statistically significant. Although many studies have noted an increased incidence of Candida colonization in

denture wearers, the number of people who wore dentures in our study was too small (n=4) to draw any statistical conclusion.

Many studies have implicated *C. albicans* to be the most common cause of colonization as well as candidiasis in cancer patients. [32, 33, 22, 20, 15] Similar results were seen in our study. *C. albicans* was the most common species causing oral colonization as well as candidiasis in cancer patients as well as the control group. Amongst the non-albicans species, *C. glabrata* and *C. tropicalis* were found to be the leading cause of colonization and infection, a trend noted by many other investigators. [48, 15, 16]

Many studies have shown an increase in the resistance to fluconazole among *C. albicans* isolates. [32, 28]However, none of the *C. albicans* isolates recovered from cases as well as controls in the present study were resistant to the first line azoles, fluconazole and voriconazole. Non-albicans species although being universally sensitive to voriconazole, showed a decreased sensitivity to fluconazole in both the groups. *C. glabrata* and *C. krusei* were found to be resistant to fluconazole as has been reported by other authors. [26**,** 28]

In conclusion oral colonization and infection by Candida spp. is a matter of concern in patients with various malignancies in our hospital. Multiple risk factors contribute to such a scenario in this vulnerable group. *C. albicans* continues to be the number one cause of oral candidiasis in cancer patients in our hospital. All the *C. albicans* recovered were found to be sensitive to the first line azoles; fluconazole and voriconazole. A high resistance pattern in non-albicans spp. to fluconazole seen in this study highlights the need for prompt identification and drug susceptibility testing of the infecting Candida spp. in cancer patients before starting empirical therapy. Also, in view of the increasing reports of resistance to first line azole antifungals, from many parts of the world, even in *C. albicans*, the need for closely monitoring the trends in the epidemiology and drug susceptibility of Candida spp. cannot be overemphasized.

Tables and figures in the paper

Risk factors	Oral Candida Colonization seen	Oral Candida Colonization absent	Total
Age > 60 years	**27(67.50%)**	**13(32.50%)***	**40(100%)**
Chronic ailments (e.g.diabetes)	**3(75%)**	**1(25%)**	**4(100%)**
Use of dentures	**4(100%)**	**0**	**4(100%)**
Recent H/O oral fungal infection	**13(81.25%)**	**3(18.75%) ** **	**16(100%)**
H/O prior surgery	**10(47.62%)**	**11(52.38%)**	**21(100%)**
Chemotherapy	**48(42.48%)**	**65(57.52%)**	**113(100%)**
Chemotherapy and Radiotherapy	**27(72.97%)**	**10(13.03%)**	**37(100%)**
Antibiotic intake	**24(57.14%)**	**18(42.86%)**	**42(100%)**

***p=0.024, **p=0.010**
Table: 1. Risk factors for oral Candida colonization in cancer patients.

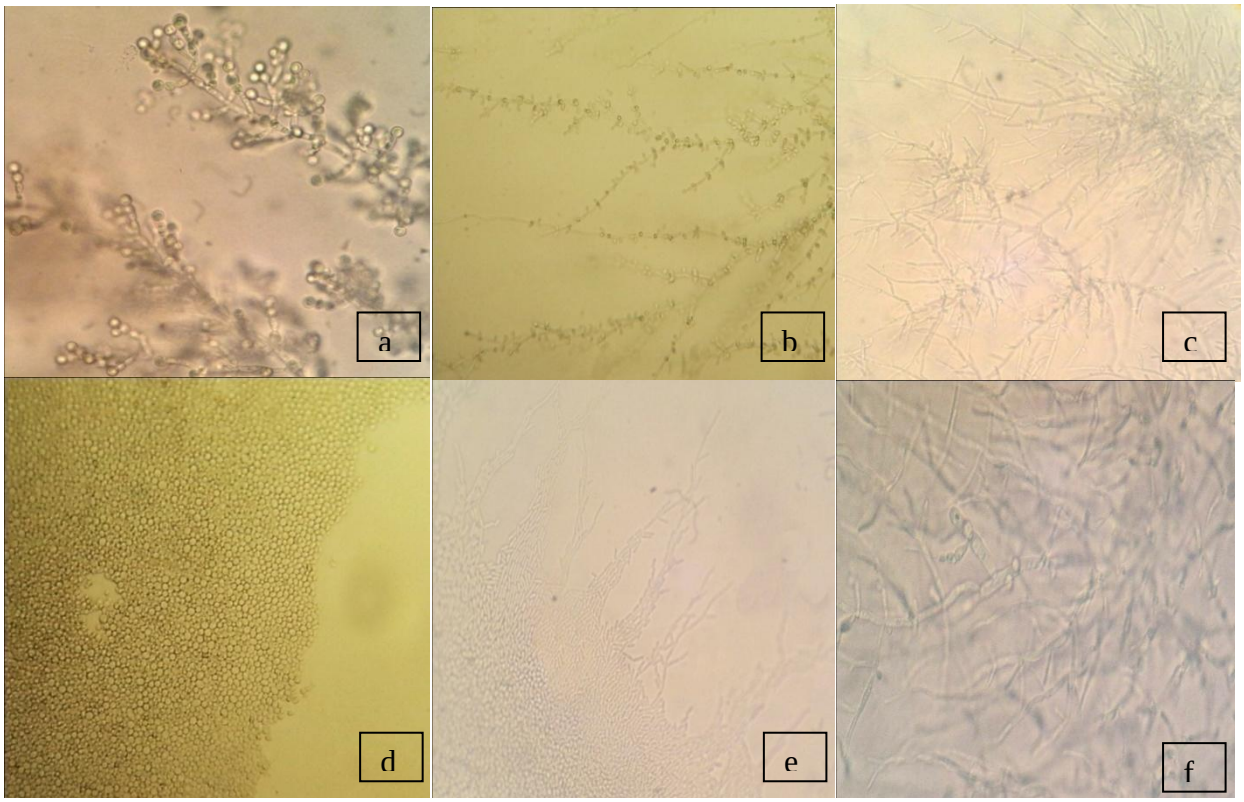


Fig-1: CMA morphology of various Candida spp. isolated. a: *C. albicans* b: *C. tropicalis* c: *C. krusei* d: *C. glabrata* e: *C. kefyr* f: *C. parapsilosis*

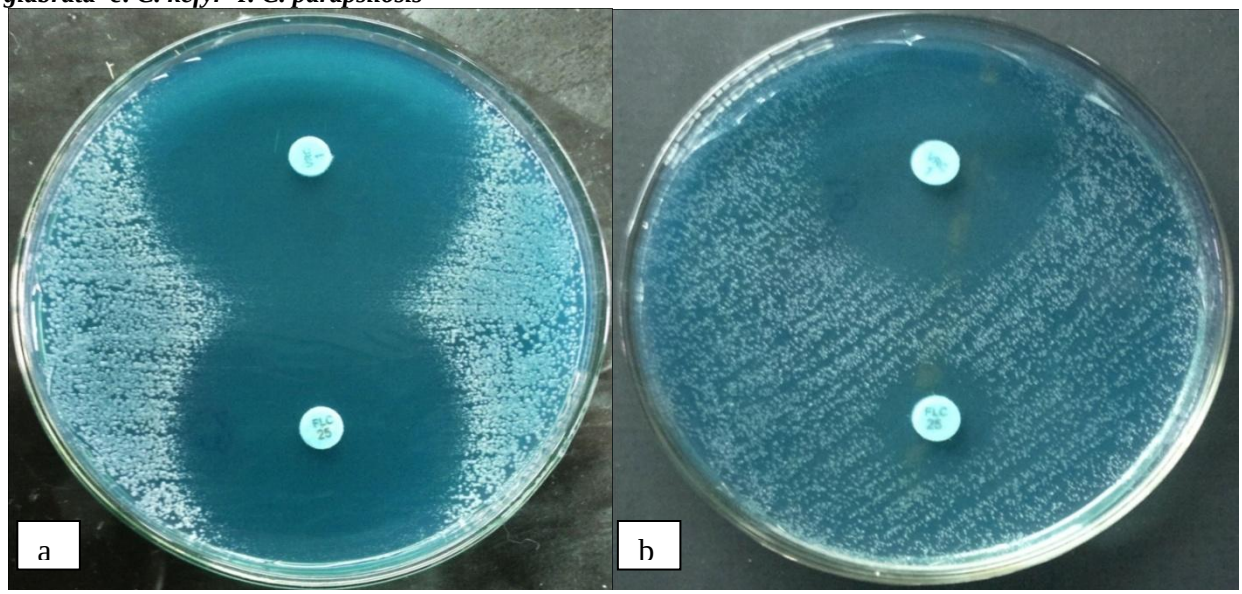
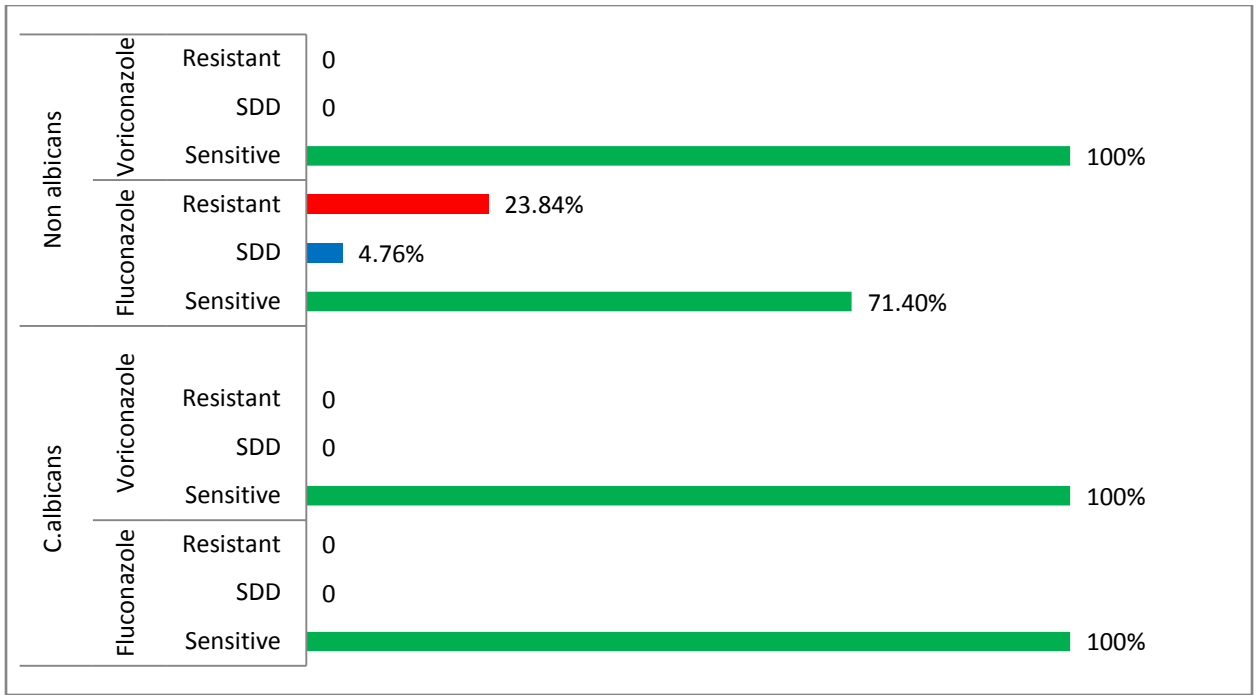
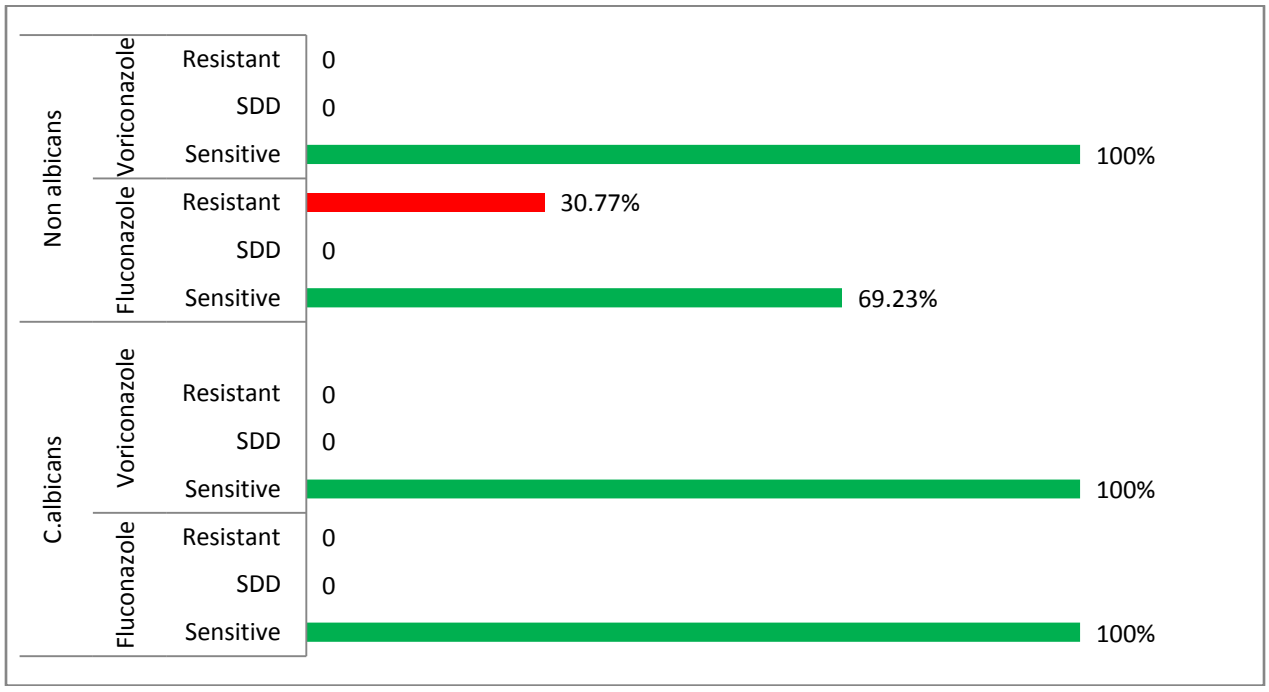


Fig- 2: Antifungal disc diffusion susceptibility testing of Candida spp.
a: Isolate sensitive to fluconazole and voriconazole
b: Isolate resistant to fluconazole but sensitive to voriconazole.



SDD: susceptible dose dependent

Fig- 3: Antifungal susceptibility profiles of Candida isolates recovered from cases.



SDD: susceptible dose dependent

Fig- 4: Antifungal susceptibility profiles of Candida isolates recovered from controls.

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