ORIGINAL RESEARCH ARTICLE

Prevalence of Non-Albicans *Candida* Among the Patients Attending a Tertiary Care Hospital in Kathmandu, Nepal

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Abstract

The main objective of this study was to determine the prevalence of non-albicans *Candida* among the patients attending a tertiary care hospital in Kathmandu, Nepal. *Candida* spp. isolated from different clinical samples (sputum, urine, vaginal swab, blood, endotracheal (ET) secretion, pus) from 250 patients between the period of February 2013 and December 2015 were included in the study. Of those 250 patients, 20% were immunocompromised. Sabouraud dextrose agar was used for the isolation of *Candida* spp. and the identification was performed on the basis of colony morphology, Gram's stain, India ink preparation, germ tube test, temperature tolerance test, characteristic color change in CHROMagar, chlamydospore production, sugar fermentation test and sugar assimilation test.

Out of total 300 Candida spp., majority were isolated from sputum (43.33%) followed by urine (40%) and vaginal swab (6.67%). Of total 151 (50.33%) non-albicans *Candida*, the most common species isolated were *C. tropicalis* (62.25%) followed by *C. glabrata* (23.84%). High prevalence of non-albicans *Candida* among the patients attending a hospital in Kathmandu, Nepal was noted.

Key words: *Candida*, non-albicans *Candida*, Nepal

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Introduction

Incidence of fungal infection has increased significantly over the past few years, causing considerable morbidity and mortality, mainly affecting immunocompromised patients. The emergence of fungal infections as the worldwide health care problems may be attributed to the extensive use of broad spectrum antibiotics and immune-suppressive agents, as well as an increase in the population of immunocompromised people [1]. *Candida* is one of the commonest fungal pathogens and may cause infection in immunocompetent as well as immunocompromised person [2]. Due to presence of *Candida* spp. as normal flora of mucosal surfaces, they mostly cause endogenous infections [3]. *Candida albicans* is considered as the commonest species of *Candida* responsible for causing various infections [4]. But in recent years there have been significant increase in infections caused by the species of the non-albicans *Candida* [5]. Only small numbers of species among the 150 species of *Candida* are well established as human pathogens [6]. Among them, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis* are non-albicans *Candida* of increasing clinical significance [5]. Most of non-albicans *Candida* like *C. glabrata, C. krusei, C. tropicalis* and *C. parapsilosis*, which are of clinical significance are known to show resistance toward certain commonly used antifungal agents [7, 8]. So, for optimizing the treatment of the infections caused by *Candida* spp., it is necessary to identify the *Candida* spp. up to species level, even if it is not possible to perform antifungal susceptibility testing [9]. Further the isolation of the *Candida* spp. from the clinical samples like sputum, urine, vaginal swab etc. does not necessarily suggest the infection and clinical correlation or confirmation of infection by alternative methods is necessary. However, the high rate of isolation of *Candida* spp. from clinical specimens (mainly from debilitated patients) suggests the possibility of high rate of endogenous infections by these organisms. In Nepal there are limited data regarding the rate of isolation of non-albicans *Candida* from different clinical specimens. So, in this study we determined the

prevalence of non-albicans *Candida* among the patients attending a tertiary care hospital.

Materials and Methods

Candida spp. isolated from different clinical samples (sputum, urine, vaginal swab, blood, endotracheal secretion, pus) from 250 patients between the period of February 2013 and December 2015, were included in the study. Of those 250 patients, 20% were immunocompromised. The clinical specimens were collected using standard techniques [10]. For the isolation of the *Candida* spp. the samples were inoculated on sabouraud dextrose agar and were incubated aerobically at 35°C for 48 hrs. Easily emulsifiable, white, opaque, dome or flat shaped colonies were subjected to further identification by Gram's stain and India ink preparation. The *Candida* spp. were identified up to species level by using the following methods:

Germ tube test: This test was used for the preliminary identification of the *Candida albicans*. A small inoculum of the yeast cells from pure culture was suspended in 0.5 ml of sheep serum and was incubated at 37°C for three hours. Then a drop of the incubated serum was observed under microscope using 40X objective. The isolates were identified as germ tube producing or germ tube non-producing.

Temperature tolerance: This test was also used for the presumptive identification for *Candida albicans*. The isolates were cultured on sabouraud dextrose agar and incubated aerobically at 45°C for 72 hours and observed for any growth if present.

CHROMagar Candida: CHROMagar Candida (HiMedia, Mumbai, India) was used for presumptive identification of various *Candida* species. The pure culture was seeded into CHROMagar media and incubated at 35°C for 48 hours. The media was observed for characteristic color change.

Chlamydospore production: It was used for the preliminary confirmatory identification of *Candida albicans*. The isolates were inoculated on corn meal agar by slide culture technique and incubated at 25°C for 72 hours and observed for chlamydospore production using lactophenol cotton blue stain.

Sugar fermentation test: Six percent solution of dextrose, maltose, lactose and sucrose with basal media were used for the test.

Sugar assimilation test: Sugars used for this test were glucose, lactose, maltose, sucrose and galactose.

Ethics statement: Our study was in compliance with Helsinki declaration.

Results

Between February 2013 and December 2015, 300 *Candida* spp. were isolated from various clinical samples. Majority of the *Candida* spp. were isolated from sputum (43.33%) followed by urine (40%) and vaginal swab (6.67%) (**Figure 1**).

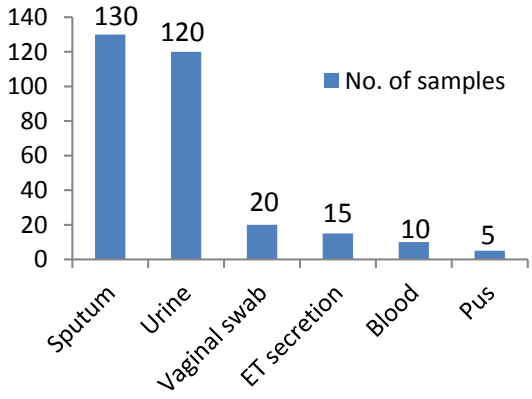

Figure 1: Sample wise distribution of *Candida* spp.

Table 1: *Candida* spp. isolated from different clinical samples.

Candida spp.	Sputum	Urine	Vaginal swab	ET secretion	Blood	Pus	Total
C. albicans	65	56	10	8	6	4	149
C. tropicalis	43	40	6	2	2	1	94
C. glabrata	10	16	3	5	2	_	36
C. kefyr	7	6	_	_	_	_	13
C. krusei	3	_	1	_	_	_	4
C. dubliniensis	2	2	_	_	_	_	4
Total	130	120	20	15	10	5	300

Among total 300 *Candida* spp. isolated, 149 (49.67%) were *Candida albicans* and 151 (50.33%) were non-albicans *Candida*. Out of 151 non-albicans *Candida* spp., *C. tropicalis* (62.25%) was the most common non-albicans *Candida* followed by *C. glabrata* (23.84%) (**Table 1**).

Discussion

In spite of their ubiquitous presence, only a few fungi are considered pathogenic. Skin, nail and hair are the commonest sites of fungal infections. But recently there have been increased numbers of systemic fungal infections due to the use of broad spectrum antibiotics, increase in the numbers of immunocompromised persons (due to conditions like lymphomas, leukemia, organ transplantation, human immunodeficiency virus (HIV) infection and use of immunosuppressive drugs) [11]. Among the fungal pathogens, *Candida* is a leading cause of a variety of human infections [2].

In our study the rate of isolation of non-albicans *Candida* (among all *Candida* spp.) was > 50%; which was in accordance to the result reported by Deorukhkar et al. (63.3%) [2]. In the present study, among total 130 *Candida* spp. isolated from sputum, the rate of isolation of *C. albicans* was 50% followed by *C. tropicalis* (33.076%) and *C. glabrata* (7.69%). Similar to our observation, in a study by Jha et al., the commonest species of *Candida* isolated from sputum was found to be *C. albicans* (70%) followed by *Candida tropicalis* (13.33%) [3]. However, the isolation of the *Candida* spp. from sputum in our study did not necessarily suggest the infection and clinical correlation or confirmation of infection by alternative method was necessary. But the isolation of the *Candida* spp. from the immunocompromised people (having predisposing risk factors) suggests the higher risk of endogenous infection. The main risk factors responsible for increased numbers of respiratory tract infections by *Candida* spp. are smoking, chronic obstructive pulmonary disease, tuberculosis, malnutrition, malignancy, diabetes mellitus, human immunodeficiency virus infection and prolonged use of antibiotics [3].

In urine samples, the majority of *Candida* spp. isolated were *C. albicans* (46.67%) followed by *C. tropicalis* (33.3%) and *C. glabrata* (19.2%). Among all *Candida* spp. the rate of isolation of non-albicans *Candida* from urine was 53.33%. Our finding was in accordance with the findings of Deorukhkar et al. [2] and Kauffmann [12], in which >50% of the *Candida* spp. isolated from urine samples were non-albicans *Candida*. Along with the well adaptability of the non-albicans *Candida* spp. for urinary tract infection, it is more difficult to treat the infection caused by them in comparison to that caused by *C. albicans* [2]. Old age, diabetes mellitus, pregnancy and urinary catheterization are the predisposing factor for urinary tract infection by *Candida* spp.

In a study by Helmy, 14 % of the cases of vulvovaginal candidiasis were due to non-albicans *Candida* [8] but in our study 50% of the *Candida* spp. isolated from vaginal swab were non-albicans *Candida*. However, in both studies the *C. tropicalis* followed by *C. glabrata* were the most common species of *Candida* isolated from vaginal swab. In the study done by Deorukhkar et al. the predominant *Candida* spp. responsible for causing vulvovaginal candidiasis were found to be *C. glabrata* followed by *C. tropicalis* [2].

Although, the isolation of the *Candida* spp. from urine and vaginal swab does not necessarily suggest the infection; the high rate of isolation of these organisms from patients having risk factors for infection by *Candida* spp. could not be neglected. In our study 20 % of the patients were immunocompromised.

All the patients from whom *Candida* spp. were isolated from endotracheal secretion were from intensive care unit and were intubated. And non-albicans *Candida* isolated from their endotracheal secretion were 43.75% of all the *Candida* spp. isolated from endotracheal secretion. This indicates that the intubation is a very important risk factor for acquiring yeast infection.

In our study, 40% of the non-albicans *Candida* were isolated from the cases of candidemia. The predominant non-albicans *Candida* isolated from blood were *C. glabrata* followed *C. tropicalis*. Among all *Candida* spp., we found *C. albicans* to be the most predominant cause of candidemia. But in the study by Deorukhkar et al. *C. glabrata* was the

commonest species isolated from the cases of candidemia [2]. Despite the isolation of *C. glabrata* (mortality rate due to infection by which is higher) from only 20% of the cases of the candidemia, in our study the mortality rate for the cases of candidemia was 80% [2].

In our study rate of isolation of *Candida* spp. was higher among patients from intensive care unit and those having history of treatment with broad spectrum antibiotics (for long period of time). The use of broad spectrum antibiotics upsets the balance of the normal bacterial flora and results in infection by *Candida* spp. [2]. Further the patients from intensive care unit are debilitated.

Due to adaptation of the *Candida* spp. to various habitats including medical devices, the incidence of hospital acquired infection due to *Candida* spp. has increased [2]. *Candida* spp. have ability to form biofilm and in the study by Deorukhkar et al. the biofilm forming capability has been found to be higher in *C. tropicalis* as compared to *C. albicans*. This attribute can help non-albicans *Candida* like *C. tropicalis* to show higher drug resistance [2]. Further, some non-albicans *Candida* are intrinsically resistant to some antifungals mainly in context of opportunistic infections in immunocompromised [4].

Conclusion

High prevalence of non-albicans *Candida* among the patients attending a hospital in Kathmandu, Nepal was noted. From our study, it can be concluded that non-albicans *Candida* may be of high clinical significance mainly in case of the patients with predisposing risk factors.

Author's Contribution

NDP and MS designed the study, performed the research work, analysed the data and prepared the final manuscript. PP contributed in analyzing of the data.

Competing Interests

The authors declare that they have no competing interests.

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