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Colonization of Health care workers by *Candida* species in Intensive Care Units of Tertiary health care centre in North Karnataka.

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ABSTRACT

Introduction: *Candida* nosocomial infection and multidrug resistance have become a major threat in health care setup. *Candida*, an opportunistic pathogen, colonizes humans at various sites like the skin, mucous membrane of the Gastrointestinal tract, Respiratory system etc. In most cases of *Candida* infection, isolated species are found to colonize the host. However, reports suggest transmission by hand of those colonized by the opportunistic pathogen and by fomites. Over recent decades, *Candida auris* has emerged as a pathogen known to cause serious nosocomial infections. The present study was carried out to identify *Candida* species colonization among various Health care workers (HCWs) in Intensive care units (ICU) and to know if *Candida auris* is among the *Candida* species isolated.

Materials and Methods: Swabs from both hands, nostrils, and oral cavity were collected from 125 healthcare workers of ICUs, at the Tertiary Health care centre of North Karnataka, between December 2022 and February 2023. The samples were cultured on Sabouraud's Dextrose agar. Morphological characterization of yeasts was done using gram stain, germ tube formation test, and Hi-Crome *Candida* differential agar.

Results: Twelve out of 125 (9.6%) volunteers were colonized with one of the 2 *Candida* species, *Candida krusei* and *Candida albicans*, at various sites. Four out of 12 volunteers were found to be colonized by *C. albicans* in the oral cavity, and 8(66%) were colonized by *Candida krusei* in the oral cavity and other sites. *C. krusei* was the predominant *Candida* species isolated from hands of the HCWs of ICU followed by *C. albicans* that was only found in the oral cavity.

Conclusion: *C. krusei* was the predominant *Candida* species isolated from HCWs of ICU followed by *C. albicans*. *C. auris* was not isolated from any of the healthcare workers. The findings suggest that surveillance for *Candida* species among healthcare workers could help in reducing nosocomial infection.

Keywords: Hand Hygiene, *Candida* colonization, Hospital infection control, Health care workers

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INTRODUCTION

Candida species are the commensals normally present on the body like skin, mucosa of gastrointestinal tract and can be found in about 60% of healthy individuals [1]. They can be transmitted from person to persons by hand and fomites. They are known to cause opportunistic infection in immunocompromised individuals such as those suffering from diabetes, leukaemia & HIV-AIDS *etc.* [2]. Most common diseases caused by *Candida* species include oral thrush, skin infection, bloodstream infection, rashes in the neonates, vaginal Candidiasis in the women, septicaemia in ICU patients *etc.*[3]. Patients with frequent or prolonged use of broad spectrum antibiotics that disrupt the resident beneficial bacterial flora, prolonged central venous catheters, foleys urinary catheter or any other invasive devices, abdominal surgery, and prolonged stays in the ICU will be at a greater risk of developing opportunistic infections due to *Candida* species [4].

Candida albicans is the most commonly isolated spp. that causes infection in humans [3][5]. Healthy humans often harbour candida at various mucosal membranes. Also, studies suggest that it has been rarely isolated from environment [6]. However, infections caused by *Candida non-albicans* species are presently more common and reports suggest it is more common in debilitated patients who were previously exposed to fluconazole [7].

Over recent years *C. auris* has emerged as a threat in healthcare settings [8]. It was first identified in 2009 from ear discharge of a 70-year-old Japanese woman [9] and since then has been isolated from various cases. In India, infection by *C. auris* was documented around 2011 through a multicentric study on candidemia cases from ICUs [10]. It is known to colonise the skin, linens and other fomites and persists in hospital environment for prolonged durations [11].

C. auris often show high level resistance to antifungal agents that can pose a risk for proper treatment especially in immunocompromised individuals [12]. Colonised patients or health care workers can be a source of infection to other immunocompromised individuals. In most

cases of infection by *Candida* species, the treatment is empirical administration antifungal drugs, however the complications surfaced when multidrug resistant strains emerged over time and identification of pathogenic strain become important [12].

Identification of *Candida auris* can be complicated as it has been misidentified using traditional microbiology methods. Previous studies reported, VITEK-2 systems, BD Phoenix misidentified *C. auris* as *C. haemulonii* or *C. glabrata*, *Microscan walkaway* misidentified it as *C. tropicalis* [13]. Studies also reported negative result on BioFire's Filmarray Blood Culture identification as the traditional panel for identification of *Candida* species did not include *C. auris*.

A person colonized with various *Candida* species may unknowingly pass the fungus to another person and may cause serious infections in immunocompromised individuals. The present study was carried out to identify *Candida* species in various health care workers in ICUs at a Tertiary health care centre of North Karnataka.

MATERIALS & METHOD

Study Participants

The study protocol was approved by the Institutional Ethics committee (Ref No. MDC/JNMCIES/5). One hundred and twenty five eligible participants were enrolled in the study after obtaining written informed consent. Persons with skin infection or on anti-fungal therapy or immunosuppressive treatment were excluded. The study participants included Doctors, Nurses, Physiotherapist, support staff and intern students from 4 different Intensive Care Units (MICU, ICCU, Liver ICU, NSICU) of a Tertiary care centre in North Karnataka. The study was conducted between the period of December 2022 and February 2023. All demographic information of participants such as age, gender, clinical signs was recorded in questionnaires.

Sample collection:

Samples were collected from the Right hand, Left hand, Right nare, Left nare & oral cavity by gently rubbing the sterile cotton swab. The sample from each site was taken with the different swab. In order to collect hand samples, sterile swabs were first dipped in sterile saline and then rubbed on both hands, paying particular attention to the spaces between the fingers and the nail beds. For oral sample, the oral vestibule & gums were gently swirled with the sterile swabs, then it was stored in sterile saline till further processing and for nasal sample, moist sterile swab was swirled around the anterior part of nose and was transported in 2 ml of sterile normal saline solution in a sterile test tube covered with cotton plug and labelled.

***Candida* isolation and identification**

The samples collected were subjected to culture and identification. All the swabs were plated on the Sabouraud dextrose agar (SDA) containing chloramphenicol. Plates that showed growth were further processed with gram staining for the confirmation of budding yeast. Germ tube formation test was performed by incubating the isolates in 0.5ml of pooled human serum at 37°C for 4 hours to detect formation of true germ tubes for detection of *Candida albicans*.

Further, all isolated yeasts were sub-cultured on chromogenic Hi-Chrome agar and incubated at 37° C for 48 hours. All *Candida* isolates were identified on the basis of different colours such as light green colour for *Candida albicans* and purple fuzzy for *Candida krusei*.

STATISTICAL ANALYSIS:

The data collected was entered into SPSS software and Analysis was done using SPSS

(Statistical Package for Social Science) software version 20.0. Descriptive statistics like Rate & Frequency was used to find the prevalence.

RESULTS

One hundred and twenty five health care workers included Doctors and intensivists (8), Nurses (66), Physiotherapist(5), support staff(16) and intern students(30); 46 were men (mean age of 27 years) & 79 were women (mean age of 26 years). Age of health care workers ranged from 19 years to 53 years.

Candida spp. grown on Sabouraud dextrose agar produced white to cream-colored colonies that were smooth and waxy. The isolated colonies were further subjected to gram staining to confirm the presence of budding yeast. Microscopy revealed gram positive spherical to ovoid yeast cells that also showed budding.

Further, germ tube formation test revealed formation of true germ tubes without a constriction at origin differentiating *C. albicans* (Figure 1.a) from non-*albicans Candida* species. (Figure 1.b)

A total of 12/125 (9.6%) health care workers were found to be colonized with *Candida* species. Out of those 12, eight were staff nurses, two were interns, one support staff and one physiotherapist. Among these 12 health care workers 9 were found to be colonised at 1 out of the 5 sites, the rest 3 were found to be colonised at 2 of the 5 sites, yielding 15 isolates. Maximum colonization was found on the hands and oral cavity of HCW as compared to nostrils as only 1 HCW was found to be colonised at right nostril. (Tables 1 and 2).

Four of fifteen isolates showed true germ tube formation and were *C. albicans* (Table 3). On the chromogenic Hi-Crome *Candida* differential agar, the 4 (4/15 *i.e.* 26%) isolates that showed positive germ tube formation, formed light green coloured colonies confirming growth of *C. albicans* (Figure 2-Q3). The remaining 11 (11/15 *i.e.* 74%) isolates that did not

form germ tube, showed a growth of purple colour fuzzy colonies (Figure 2.a-Q1, Q2 & b) indicating growth of *C. krusei*. (Table 3)

DISCUSSION

Candida species over the years have become an important cause of infections in health care settings especially in those who are immunocompromised leading to profound morbidity and mortality [15]. Risk factors for development of candida infections include prolonged use of broad spectrum antibiotics, malignancies, immunosuppression, uncontrolled diabetes, prolonged hospitalization, major surgical procedures, long standing catheters or central lines etc. *Candida albicans* is the most frequently isolated species from cases of candidemia [5]. However, recent trends show emergence of non-*albicans Candida* species causing more serious infections in ICU settings with greater morbidity and mortality as compared to *C. albicans* which is attributed to increasing use of antifungal agents especially Fluconazole [13].

Usually, it is thought that the endogenous flora colonising the skin or mucous membrane of the patient is responsible for the opportunistic infections [16]. However, there are reports to suggest that *Candida* species can be acquired exogenously from other persons harbouring the pathogen or from contaminated environment. Following exposure, it is found to survive around 45 minutes on hand and on various other surfaces for up to 4 months in hospital environments [17].

Studies suggest that about 60% of HCW are colonised by various candida species [18]. The contaminated hands of an asymptomatic carrier can be a source of infection in a susceptible host. The aim of this study was to identify various *Candida* species colonizing the health care workers of ICU.

Twelve (9.6%) out of 125 health care workers in this study were found to be colonized with *Candida* species. In a study by Karina Mayumi Sakita et al (2017), samples were

collected for detection of *Candida* colonization from hands of 57 HCW of ICU. Thirty six of the 57(63.15%) health care workers were found to be colonised by various *Candida* species [19]. Ralciane de Paula Menezes et al (2018) conducted an NICU based study where 37.3% (50/134) of HCW were colonised by various *Candida* species [18]. The findings of the above mentioned studies are higher prevalence of *Candida* colonization than that observed in the present study.

A study by Harsimran Kaur et al (2018), conducted in a paediatrics unit located in Chandigarh, 40 environmental samples and samples from both hands of 24 HCW were collected. One HCW was found to be colonized by *C. krusei*. Other species isolated included *C. tropicalis* ($n = 11$), *C. albicans* ($n = 6$), *C. parapsilosis* ($n = 3$) and *C. glabrata* ($n = 2$) from environment as well as hands of HCW. Unfortunately authors have not specified the number of hand colonization of HCW for the above isolates [20]. Another study by Vishak C Keri et al (2021) showed a 5% colonization of hands of HCW in medical wards and ICU [21], and the observed colonization rate is slightly lower than that observed in present study.

Most of the HCW in present study were found to be colonized in mouth (40%) followed by right hand and left hand (27%). Compared to hands and oral cavity, the nostrils were less frequently colonized with *Candida* species. Similarly, in other study, it is justified that *C. albicans* are usually found to colonize the mucosal lining, and can also be transported by the hands along with other species to other sites and also to patients as well.

In the present study, two *Candida* species *i.e.* *C. krusei* and *C. albicans* were identified using Hi-Crome *Candida* differential agar, among which *C. krusei* (73%) was predominant as compared to *Candida albicans* (26%). *C. krusei* was isolated from all the sample sites and also was the most common species to be isolated from hands of HCW. All the *C. albicans*

isolates were from oral mucosa. Karina Mayumi Sakita et al (2019) [19] and Ralciane de Paula Menezes et al (2018) [18], isolated *C. parapsilosis* as the predominant species from hands of health care workers which is different from that observed in the present study. Studies suggest that, *C. parapsilosis* followed by *C. albicans* are the most common species to colonize skin [22], however findings differ from the present study; we found predominant isolation of *C. krusei* on skin.

Candida auris infection has emerged as a global problem & infections are becoming more common in hospitals and Intensive Care Units globally and even in Indian Health care settings. In a 2021 study by Vishakh C Keri et, 3/60 (5%) HCW were colonized by *Candida* species on hands that included *C. auris*, *C. tropicalis*, and *C. parapsilosis* [21]. In another study by Biswal, M et al 2017, 4/145 (2.8%) HCW of ICU were found to harbour *C. auris* [23]. The authors also state that transient colonization of hands of Health care workers can be a source of infection for the critically ill patients [21][23]. In the present study, none of the health care workers were found to be colonized by *C. auris* at any of the site

According to the literature the lack of hand hygiene of health care workers is the main cause of transmission of microorganisms [18]. Hence, if the colonized people follow all the protocols and maintain proper hand hygiene, they might not transmit it to the patients and prevent outbreaks.

CONCLUSION

In this study, it was found that *Candida krusei* (73%) and *Candida albicans* (26%) were the predominant *Candida* species isolated in the health care setting from various health care workers. *Candida auris* was not isolated from any participant. However, surveillance for *C. auris* among health care workers is needed. Since *Candida* infection occurs in immunocompromised individuals in ICUs, proper hand hygiene practices should be followed

at all-time especially the five moments of hand hygiene should be strictly followed

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Conflicts of interest: The authors declare conflict of Interest as None.

Authors' contributions: Madhuri Savant (MS): carried out the experiment, Laxmi Deshpande (LD): developed the concept and study design and Guruprasad Antin (GA): helped with the clinical material. The manuscript has been reviewed and approved by all authors, the requirements of authorship have been met, and the manuscript represents honest work.

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Tables

Table 1. Characteristics of the 125 study participants.

| GROUPS | FREQUENCY (125) | PERCENTAGE (%) | COLONISED INDIVIDUALS (12) |
|-------------------|------------------------|-----------------------|-----------------------------------|
| Age group | | | |
| 15-25 | 70 | 56.0 | 4 |
| 26-36 | 39 | 31.2 | 6 |
| 37-47 | 14 | 11.2 | 2 |
| 48-58 | 2 | 1.6 | - |
| Sex | | | |
| Male | 46 | 36.8 | 4 |
| Female | 79 | 63.2 | 8 |
| Occupation | | | |
| Doctor | 6 | 4.8 | - |
| Intensivist | 2 | 1.6 | - |
| Physiotherapist | 5 | 4.0 | 1 |
| Staff nurse | 66 | 52.8 | 8 |
| Interns | 30 | 24.0 | 2 |
| Support staff | 16 | 12.8 | 1 |

Note: Observations of various characteristics of the colonized health care workers.

Table No. 2: *Candida* species and sites of colonization

| Clinical swab samples | <i>Candida</i> species isolated | |
|-----------------------|---------------------------------|-----------------------|
| | <i>Candida albicans</i> | <i>Candida krusei</i> |
| Oral (n=6) | 4 | 2 |
| Right hand (n=4) | 0 | 4 |
| Left hand (n=4) | 0 | 4 |
| Right nare (n=1) | 0 | 1 |
| Left nare (n=0) | 0 | 0 |
| Total n=15 (%) | 4(26%) | 11(73%) |

Note: *C.albicans* was found to colonise only at the oral cavity were as, *C. krusei* colonised oral cavity as well as other sites.

Table 3: *Candida* isolates obtained from various health care workers

| Sl.No | Code of participants | Right hand | Left hand | Right nostril | Left Nostril | Oral |
|-------|----------------------|------------------|------------------|------------------|--------------|--------------------|
| 1 | 5 | - | <i>C. krusei</i> | - | - | <i>C. albicans</i> |
| 2 | 10 | - | <i>C. krusei</i> | - | - | |
| 3 | 35 | - | - | - | - | <i>C. albicans</i> |
| 4 | 42 | - | - | - | - | <i>C. albicans</i> |
| 5 | 43 | <i>C. krusei</i> | - | - | - | - |
| 6 | 45 | <i>C. krusei</i> | <i>C. krusei</i> | - | - | - |
| 7 | 51 | <i>C. krusei</i> | - | - | - | - |
| 8 | 68 | - | - | <i>C. krusei</i> | - | - |
| 9 | 95 | <i>C. krusei</i> | <i>C. krusei</i> | - | - | - |
| 10 | 96 | - | - | - | - | <i>C. krusei</i> |

| | | | | | | |
|----|-----|---|---|---|---|--------------------|
| 11 | 104 | - | - | - | - | <i>C. krusei</i> |
| 12 | 113 | - | - | - | - | <i>C. albicans</i> |

Note: Only 12 people were found to be colonized with 2 types of Candida species.

Figures and Legends

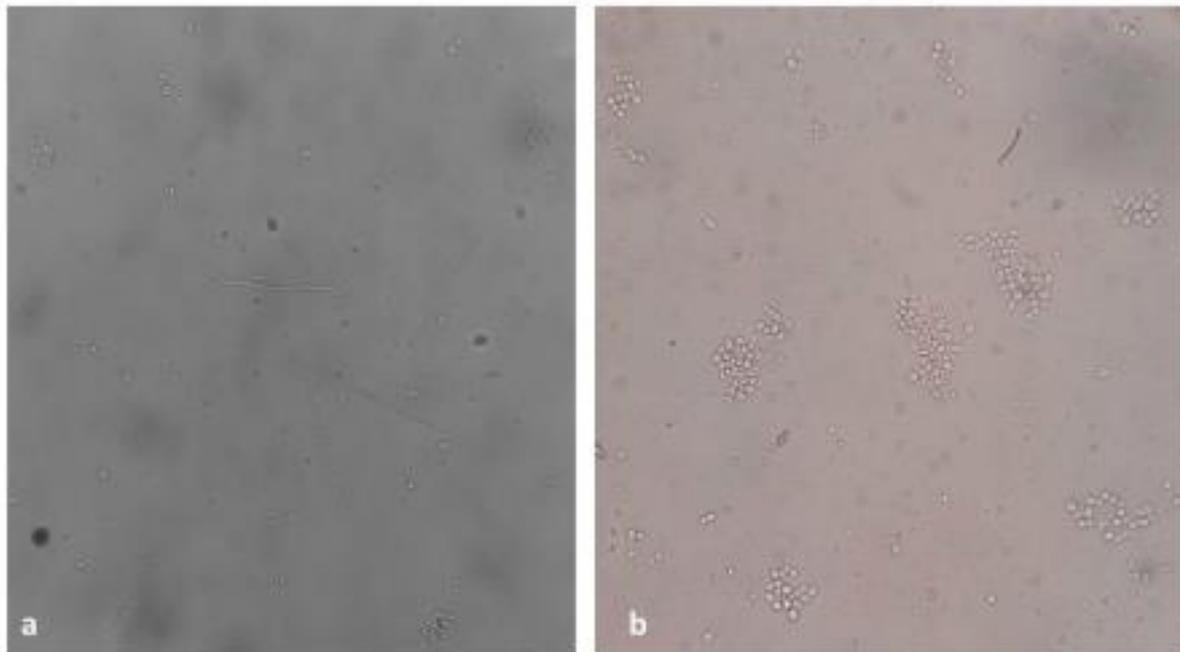


Figure 1a. Shows isolates forming true germ tube formation.

Figure 1b. Shows isolate negative for germ tube formation.



Figure 2a. Q1 and Q2 *C. krusei* forming purple colour fussy colonies. Q3: Growth of *C. albicans* forming light green colour colonies

Figure 2b. *C. krusei* forming purple colour fussy colonies.