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Burden and Sources of Fungal Contamination in ICU Settings: A Cross-Sectional Analysis

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ABSTRACT

Background: Fungal contamination in intensive care units (ICUs) poses a risk for nosocomial infections. Healthcare workers, attendants, and environmental surfaces may harbor opportunistic fungi, particularly *Candida* species. However, *Aspergillus* spp. is primarily an environmental pathogen and does not colonize healthy humans, representing transient exposure rather than true carriage. To determine high-risk sources that may contribute to nosocomial transmission, this study evaluated the prevalence of fungal contamination in a tertiary care hospital.

Methods: This cross-sectional observational study was conducted in the ICU settings of Central Referral Hospital, Gangtok, Sikkim, India, from June 2022 to March 2023. One sample per individual was obtained from consenting healthcare workers, patients' attendants who were present for at least 30 minutes in the ICU, and selected ICU surfaces from the occupied patients' bedrails. Fungal isolation and identification was performed using standard culture methods; the chi-square test was used for analysis.

Results: A total of 150 samples were collected, from healthcare workers (60; 40%), patients' attendants (50; 33.3%), and ICU surfaces on the patients' bed rails (40; 26.6%). Overall, fungal growth was detected at 76/150 (50.6%). Positivity was 28/50 (56%) among attendants, 30/60 (50%) among healthcare workers, and 18/40 (45%) on surfaces. There was no significant difference observed between the three groups (χ^2 = 1.14, p = 0.56). Among healthcare workers, housekeeping staff showed a significantly higher rate of fungal carriage than nurses (15/19 [78.9%] vs. 12/35 [34.3%]; OR = 6.75; p = 0.004), whereas the difference between nurses and interns was not significant (p = 0.64). The predominant fungal isolates were *Candida albicans* and non-*albicans Candida,* at 28/76 (36.87% each), with *Candida albicans* most common among healthcare workers and patients' attendants, while non-*albicans Candida* predominated among housekeeping staff and on environmental surfaces.

Conclusions: This study demonstrates substantial fungal contamination among healthcare workers, patients' attendants, and ICU surfaces, with *Candida* species predominating and higher contamination observed among housekeeping staff. These findings indicate contamination rather than transmission risk, underscoring the need for continued adherence to infection prevention and environmental control practices in critical care settings. The isolation of *Aspergillus* species represents transient contamination rather than a major route of transmission.

Keywords*: Fungal contamination, Candida albicans, non-albicans Candida, ICU, healthcare workers, Sikkim.*

BACKGROUND

Intensive care units (ICUs) represent high-risk environments for the transmission of healthcare-associated infections (HAIs), particularly among critically ill and immunocompromised patients. Of every 100 patients in acute care hospitals, seven patients in high-income countries (HICs) and 15 patients in low- and middle-income countries (LMICs) acquire at least one health-care associated infection during their hospital stay [1,2].Up to 30% of patients in intensive care can be

affected by health care-associated infections, with an incidence that is two to 20 times higher in LMICs than in HICs [2,3]. In India, fungi are estimated to account for approximately 5–15% of healthcare-associated infections in intensive care settings, with candidemia being the predominant fungal HAI. Multicentric Indian ICU studies have reported an incidence of 6.51 ICU-acquired candidemia cases per 1,000 ICU admissions, predominantly due to non-albicans Candida species [4, 5]. An annual report on Antimicrobial Resistance Research and Surveillance Network-2024 from the Indian Council of Medical Research (ICMR) suggests that, among healthcare-associated bloodstream infections, fungal infections accounted for 10.2% [6].Fungal infections, especially those caused by opportunistic organisms, have emerged as a significant contributor to morbidity and mortality in such settings, often leading to prolonged hospital stays and increased healthcare costs [7, 8]. Among these, *Candida* species are the most frequently implicated pathogens, responsible for a substantial proportion of invasive fungal infections in hospitalized patients [9].

The hospital environment plays a crucial role in the transmission dynamics of fungal pathogens. Environmental surfaces, medical equipment, and air systems can serve as reservoirs for fungal spores, facilitating indirect transmission to susceptible individuals [10]. Additionally, healthcare workers (HCWs) and patients' attendants may act as vectors for transient carriage of fungi, contributing to cross-contamination within ICUs [11]. Hand hygiene practices and adherence to infection prevention protocols are therefore critical in limiting the spread of these organisms [12].

Candida species typically colonize human skin and mucosa, while *Aspergillus* species mainly originate from the environment. *Aspergillus* spp. are ubiquitous molds found in air, dust, and construction areas, and their presence in hospital settings is often linked to environmental contamination rather than true colonization in healthy individuals [13]. Nonetheless, exposure to airborne *Aspergillus* spores can result in severe infections, particularly invasive aspergillosis, among immunocompromised patients in ICUs [14].

The systematic review and meta-analysis conducted in 2021 suggest that colonization significantly increases the risk of invasive candidiasis; the colonization was present in ~40% of ICU patients studied, and the pooled odds ratio is ≈ 3.3 for developing an invasive infection. This study is one of the strongest quantitative demonstrations that suggests carriage is not benign; it predicts infection risk [15].

Healthcare staff's hands are a significant source of fungal infections, and simple hand washing can greatly reduce the risk of nosocomial infections. A study at AIIMS, New Delhi, found fungal carriage rates of 33.3% among personnel, 5% on hands, and 28.3% on aprons and scrubs [16]. Healthcare workers act as conduits of transmission via contaminated hands and belongings [16].

Therefore, the present study aimed to assess the burden of fungal contamination among healthcare workers, patients' attendants, and ICU environmental surfaces in a tertiary care hospital and to identify high-risk sources contributing to potential nosocomial transmission.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional observational study was conducted in the different Intensive Care Units (ICUs), including the Medical ICU (10-bedded), Surgical ICU (six-bedded), Neonatal ICU (five-bedded), and Pediatric ICU (six-bedded) of a 500-bedded tertiary care hospital in Sikkim, India, over a period of ten months (June 2022 to March 2023). This study aimed to assess fungal contamination and high-risk sources among healthcare workers (HCWs), patients' attendants, and surfaces within the ICU environment.

Participant Recruitment

Participants were selected using random sampling from among HCWs working in the ICUs (nurses, interns, and housekeeping staff) and patients' attendants present in the ICUs during the study period. Patients' attendants or attendees are relatives who are constantly present with the patient in the ICUs. The informed written consent was obtained prior to sample collection. Inclusion criteria: Participants who were present in the ICU for at least 30 minutes prior to sample collection and provided informed consent. No restrictions were applied based on the timing of prior hand hygiene practices. Environmental sampling was conducted on ICU surfaces, specifically bedrails of patient-occupied beds at the time of sample collection. The timing of the most recent surface disinfection was not considered during sampling. Initially sampled, other ICU surfaces and the mobile phone samples are excluded from the final analysis due to procedural inconsistencies and potential contamination risks that could affect data comparability.

Ethical clearance: The study was conducted after obtaining institutional ethics committee clearance in accordance with the Declaration of Helsinki and after taking verbal and written consent from the study participants/their guardians.

Sample Collection

Samples were collected from:

- Health care workers: From the healthcare workers (nurses, interns, and housekeeping staff), one sterile moist swab sample was collected from each participant's palmar aspect of the hand.
- Patients' attendants: From patients' attendants, one sterile moist swab sample was collected from each participant's palmar aspect of the hand.
- One sterile, moist swab sample was collected from the bed rail of each patients' bed.

Sampling was performed using sterile swabs moistened with sterile saline. No hand hygiene or surface cleaning was performed immediately prior to sampling, to reflect real-time carriage and contamination levels. The timing of sample collection relative to the last hand hygiene was not recorded, which is acknowledged as a limitation. The sample was taken only once, so there is no frequency to follow. The collected samples were transported immediately under aseptic conditions to the laboratory for further processing.

Fungal Isolation and Identification Swabs were inoculated onto Sabouraud Dextrose Agar (SDA) with Chloramphenicol and incubated at 25°C and 37°C for up to 20 days. Every alternate day, the cultures were observed. As the growth appeared, it was identified based on colony morphology and microscopic features using Lactophenol Cotton Blue staining.
Species-level identification of *Candida* spp. was performed using CHROMagar Candida and Germ tube tests. *Aspergillus* spp. was identified based on macroscopic and microscopic characteristics following slide culture.

Statistical analysis: Fungal carriage rates were calculated as the proportion of positive samples among all collected samples and reported with 95% confidence intervals (95% CI)**.** Comparisons between groups (healthcare workers vs. attendants; nurses vs. housekeeping staff) were performed using the Chi-square test (or Fisher's exact test where appropriate). The magnitude of associations was assessed using odds ratios (OR) with corresponding 95% confidence intervals. A p-value of <0.05 was considered statistically significant. All analyses were performed using SPSS version 20.

RESULTS

A total of 150 samples were collected, 60 were from60 HCWs' hands: 35 nurses, six interns, and 19 housekeeping staff, 50 samples were from 50 patients' attendants' hands, and 40 samples were from 40 bed rails.

Fungal Isolation

Fungal growth was detected in **50.6% (76/150)** of the total samples. Among healthcare workers, **50% (30/60)** of the samples showed fungal growth. Within this group, positivity was observed in **34.3% (12/35)** of nurses, **78.9% (15/19)** of housekeeping staff, and **50% (three/six)** of interns.

Among patients' attendants, fungal growth was observed in **56% (28/50)** of samples. Environmental sampling from ICU bed rails showed a **45% positive rate (18/40)**.

Comparison across the three major groups (healthcare workers, attendants, and environmental surfaces) showed no statistically significant difference ($\chi^2 = 1.14$, df = 2, p= 0.56).However, a statistically significant difference was observed among healthcare worker subgroups ($\chi^2 = 9.25$, df = 2, p = 0.0098). Post-hoc analysis revealed that fungal growth was significantly higher among housekeeping staff than among nurses (p = 0.004; Bonferroni-adjusted p = 0.012), whereas differences between nurses and interns (p = 0.64) and between housekeeping staff and interns (p = 0.29) were not statistically significant. The sample source and the isolate details are provided in Table 1

Table 1: Distribution of isolates from different sample sources

Organism → / Sampled from ↓		*Candida albicans*(%)	NAC (%)	*Aspergillus flavus*(%)	*Aspergillus fumigatus*(%)	*Aspergillus niger*(%)	*Aspergillus fumigatus* & *Aspergillus niger*(%)	Unidentified fungus (%)	Total fungi isolated(%)	No Fungus isolated (%)	Total
HCW	Nurses	5/12 (41.6)	2/12 (16.6)	0	3/12 (25)	0	0	2/12 (16.6)	12/35 (34.2)	23/35 (65.7)	35
	Interns	1/3 (33.3)	1/3 (33.3)	0	1/3 (33.3)	0	0	0	3/6 (50)	3/6 (50)	6
	Housekeeping staffs	4/15 (26.6)	6/15 (40)	1/15 (6.6)	1/15 (6.6)	1/15 (6.6)	1/15 (6.6)	1/15 (6.6)	15/19 (79)	4/19 (21)	19
Patients' attendees		12/28(42.8)	11/28 (39.2)	1/28 (3.6)	1/28 (3.6)	2/28 (7.1)	0	1/28 (3.6)	28/50 (56)	22/50 (44)	50
Environmental samples		6/18 (33.3)	8/18 (44.4)	2/18 (11.1)	2/18 (11.1)	0	0	0	18/40 (45)	22/40 (55)	40
Total		28/76 (36.8)	28/76 (36.8)	4/76 (5.3)	8/76 (10.5)	3/76 (3.9)	1/76 (1.3)	4/76 (5.3)	76/150 (50.6)	74/150(49.3)	150

NAC: Non-albicans *Candida*; Values are expressed as number/total number (%). Statistical analysis was performed using the Chi-square test.

The most frequently isolated fungi were *Candida albicans* and non-albicans *Candida*, each accounting for 36.87% (28/76), followed by *Aspergillus fumigatus* at 10.5% (8/76). Among healthcare workers, *Candida albicans* was the predominant isolate (33.3%, 10/30). It was most identified among nurses (41.6%, 5/12), whereas non-albicans *Candida* predominated among housekeeping staff (40%, 6/15). Similarly, *Candida albicans* was the most common isolate among patients' attendants (42.8%, 12/28), while environmental surfaces showed a higher proportion of non-albicans *Candida* (44.4%, 8/18).

DISCUSSION

The present study demonstrated a high overall prevalence of fungal contamination (50.6%) across healthcare workers, patients' attendants, and ICU environmental surfaces, indicating widespread dissemination of fungal organisms within the hospital environment. Although higher contamination rates were observed among patients' attendants (56%) and HCWs (50%) compared to environmental surfaces (45%), these differences were not statistically significant ($\chi^2 = 1.14$, $p = 0.56$). This suggests that fungal contamination is not confined to a specific reservoir but is distributed across multiple sources in the healthcare setting. The higher positivity among patient attendants could be due to their outdoor origin. The attendants act as a bridge between the external environment and the sterile or controlled indoor hospital environment.

The observed prevalence is comparable to findings from Indian and other regional studies reporting substantial microbial and fungal contamination in hospital environments. Studies from tertiary care hospitals in India have documented hand contamination among HCWs ranging from approximately 30% to 60%, depending on ward type, patient load, and infection control practices [17, 18]. Similarly, environmental surveillance studies in ICU settings have reported fungal contamination on frequently touched surfaces and equipment, reinforcing their role as reservoirs for healthcare-associated pathogens [19]. The prevalence observed in the present study is within the upper range of these reports, possibly reflecting differences in environmental conditions such as temperature, humidity, ventilation, and adherence to infection prevention measures.

A key finding of this study was significantly higher fungal contamination among housekeeping staff (78.9%) than among nurses (34.3%), as indicated by post-hoc analysis. This finding is consistent with published literature indicating that environmental service (housekeeping service) workers are at increased risk of microbial contamination due to their frequent contact with contaminated surfaces, waste materials, and hospital environments [17, 18].Indian studies have similarly highlighted that housekeeping personnel may have higher rates of hand contamination than clinical staff, likely due to differences in training, awareness, and compliance with hand hygiene practices [18]. This underscores the need for targeted infection prevention interventions, including structured training programs and reinforcement of standard precautions for this group.

The microbiological profile in the present study showed that *Candida* species were the most frequently isolated organisms, with *Candida albicans* and non-albicans *Candida* (NAC) together accounting for the majority of isolates. This finding is consistent with epidemiological data from India and other regions, where *Candida* species are among the leading causes of healthcare-associated fungal infections, particularly candidemia in critically ill patients [9]. The predominance of *Candida* species in hand samples supports the role of direct contact transmission in their spread, as these organisms are part of the normal human microbiota and can be transmitted via the hands of HCWs and caregivers.

In contrast, *Aspergillus* species were isolated less frequently. The pathogenic mechanisms of *Candida* and *Aspergillus* differ significantly. *Candida* infections are primarily associated with endogenous colonization and contact transmission, whereas invasive aspergillosis results predominantly from inhalation of airborne conidia rather than direct contact spread [20]. Regional studies from India have demonstrated the presence of *Aspergillus* spores in hospital air and dust samples, particularly in ICUs and construction-affected areas, emphasizing the importance of airborne transmission in aspergillosis [21]. Therefore, the isolation of *Aspergillus* species from the hands of HCWs, attendants, or environmental surfaces may represent transient contamination rather than a major route of transmission.

Environmental surfaces in the present study showed a 45% fungal contamination rate,, supporting their role as potential reservoirs for fungi. This finding is consistent with studies demonstrating that frequently touched surfaces such as bed rails, monitors, and bedside equipment can harbor microorganisms and contribute to indirect transmission via hand contact [19]. In Indian hospital settings, environmental contamination has been linked to inadequate cleaning practices, high patient turnover, and suboptimal disinfection protocols [19, 21]. However, while surface contamination is important for *Candida* transmission, its role in *Aspergillus* transmission is relatively limited compared to airborne spread.

The absence of statistically significant differences among the major groups, despite relatively high contamination rates, may be attributed to overlapping confidence intervals and limited sample sizes. The relatively small number of interns also resulted in wide variability in estimates. Additionally, the presence of unidentified fungal isolates highlights the limitations of conventional identification methods and the potential need for molecular diagnostic techniques to improve species-level characterization.

Overall, the findings of this study are consistent with Indian and regional literature, indicating that fungal contamination is common in hospital environments, with *Candida* species playing a dominant role in contact-mediated transmission. The significantly higher contamination among housekeeping staff emphasizes the need for targeted infection control

strategies, including focused training, supervision, and reinforcement of hand hygiene compliance. While hand hygiene remains central to preventing the transmission of *Candida* and other contact-transmitted organisms, preventing *Aspergillus* related infections requires strengthening of environmental control measures such as ventilation, air filtration, and dust control, in high-risk areas like ICUs.

Future studies with larger sample sizes, multicentric designs, and molecular identification of fungal isolates are recommended to further clarify the epidemiology, transmission dynamics, and clinical implications of fungal contamination in healthcare settings in India and similar resource-limited environments.

LIMITATIONS AND IMPLICATIONS

Regarding the technical aspect of the study, one of the limitations is that the day of sample collection and the patient's hospital day were not considered, as the patients' attendants may have become fungal carrier from the hospital environmental source. The timing of sample collection relative to the last hand hygiene was not recorded, which is acknowledged as a limitation. Study's small sample size, particularly the small number of interns, has also limited the findings. For moulds such as *Aspergillus*, airborne exposure is generally considered the primary route of hospital acquisition. Since the study did not include air sampling, ventilation assessment, or environmental airflow evaluation, this has limited to confirm surface-mediated transmission as a major pathway. Fungal identification relied mainly on morphological characteristics and basic phenotypic tests. There are limitations of this approach particularly regarding the identification fungi. The exclusion of certain initially collected surface and mobile phone samples may have reduced representation; however, this decision was made to preserve methodological consistency and analytical reliability. The other limitation is that the study included heterogeneous sampling categories, including healthcare workers, attendants, and environmental surfaces, which represent distinct epidemiological sources. Although findings were interpreted according to sampling source, this heterogeneity may limit the strength of epidemiological inferences and preclude conclusions regarding direct transmission dynamics. Despite all these limitations, the study provides valuable insights into fungal carriage and contamination dynamics in a resource - limited setting.

CONCLUSION

This study demonstrates a high prevalence of fungal contamination among healthcare workers, patients' attendants, and ICU surfaces, with *Candida* species being the predominant isolates. Housekeeping staff exhibited significantly higher levels of contamination than nurses, suggesting an outdoor origin, and potential differences in exposure or adherence to hygiene practices. However, these findings reflect the presence of contamination only and do not establish a direct link to nosocomial fungal infections, as no clinical correlation, epidemiological linkage, or environmental air sampling was performed. Nevertheless, the results highlight the importance of maintaining robust infection prevention and control practices, including hand hygiene, environmental cleaning, HEPA filtration, and monitoring of ventilation and humidity in critical care settings.

DECLARATIONS

- Ethical clearance: The study received ethical approval from the institutional research and ethics committee; SMIMS IRC Registration No.: IRC/2022-73 and SMIMS IEC Registration No.: SMIMS/IEC/2022-53.
- Consent to participate: The study was conducted after taking written consent from the study participants/ their guardians in the language of their understanding.
- Consent for publication: Not applicable
- Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on request.
- Competing interest: The authors declare that they have no competing interests.
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- Author's contribution: Priya Kumari conceptualized the study, and was involved in sample collection, and Rekha Sharma did the laboratory work and drafted the manuscript. Both authors contributed to the final preparation of the manuscript.
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