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ORIGINAL ARTICLE

Identification of fungal agents isolated from burn lesions using mycological and molecular methods in patients admitted to Velayat burn hospital in Rasht city during 2022-2023

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ABSTRACT

Background and Objectives: Fungal burn wound infections (FBWIs) are one of the most disastrous complications in burn patients. The present study investigated the incidence and the species distribution of fungal agents isolated from burn lesions and reviewed the feautures, underlying conditions, and outcomes of patients.
Materials and Methods: The wounds were swabbed and cultured on Sabouraud Dextrose Agar with chloramphenicol medium. Fungal identification was performed using internal transcribed spacer (ITS) and beta-tubulin sequencing.
Results: A total of 380 swab specimens were obtained. Of these, 101 patients (26.75 %) were positive in culture. Among the 101 positive cases, most isolates were from males (n= 68, 67.33%) and most of them were over 30 years old. Flame (n=38, 37.63%) was the predominant cause of burns, and previous history of ICU admission (n=35, 34.66%), presence of central venous catheter (n=25, 24.75%), and diabetes mellitus (n=17, 16.83%) were the main underlying conditions. *Candida parapsilosis* complex (n=36, 35.64%), and *Pichia kudriavzevii (C. krusei)* (n=8, 7.92%) represent the most commonly isolated species Also, 2 out of 101 patients (2%) died.
Conclusion: In the present study, non-albicans *Candida* species were much higher frequent than *C. albicans* with most cases associated with *Candida parapsilosis* complex.

Keywords: Wound infection; Burns; *Candida parapsilosis* complex; *Pichia kudriavzevii (Candida krusei)*; Iran

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INTRODUCTION

Fungal infections due to burns remain the main complication limiting the survival of patients with severe burns (1). These infections constitute the most disastrous form of trauma worldwide (2). Wound infections, candidemia, aggressive soft tissue infections, and opportunistic infections may be caused by fungal strains isolated from burn patients (1-3).

Risk factors for fungal infection in burn wounds include age of burn patients, open dressing, overall size of the burn, prolonged hospital stay, weakened immune system, large wound surface area (30-60%), late surgical excision, artificial dermis, broad-spectrum antibiotic therapy, and steroid therapy (4).

Due to lack of clinical awareness, similar presentation to bacterial infections, and lack of mycology laboratories, especially in Iran the majority of fungal infections are overlooked (5). On the other hand, diagnostic performance can sometimes be delayed because clinical signs are not very specific and it is difficult to differentiate between colonization and infection (5).

Any rapid change in the burn wound appearance including conversion of a partial-thickness injury to full-thickness injury, or loss of previously viable tissue or skin graft or change in the clinical condition of the burn patient may herald burn wound infection or sepsis and prompt diagnosis and treatment of these fungal diseases can save lives (6).

The most common organisms causing burn wound infections remain *Staphylococcus* and *Pseudomonas*; however, the epidemiology of burn infections has changed over time and is geographically dependent (7). Nowadays, the incidence of fungal wound infections in burn patients has increased since the use of topical antibiotics to control bacterial colonization (8). *Candida* species remain the main cause of fungal wound infections in burns. The emergence of drug resistance in non-albicans *Candida* spp., the unusual yeasts, and molds in burn wound infections, make very few fungi sensitive to antifungals (1). Filamentous fungi (also known as molds) are known to cause invasive wound infections in patients with extensive burns (9). Diagnosing and treating invasive mold infections is challenging because filamentous fungi have much greater invasive potential than yeasts (10). It is important to know the flora of the organisms in each burn unit to be able to effectively treat burn wound infections. There is limited information in the literature about the incidence and the species distribution of fungal agents isolated from burn lesions in Iran. The main objective of the present study was to evaluate the incidence and the species distribution of fungal agents isolated from burn lesions in our burn center. Secondary objectives were to review characteristics, risk factors, and outcomes of patients with such lesions.

MATERIALS AND METHODS

Ethics statement. Informed consent has been obtained from patients or their guardians prior to sampling. This study was approved by ethical committee of Tehran University of Medical Sciences (the number of Ethics Committee protocol: IR.TUMS.SPH.REC.1401.017).

Sampling and data collection. This descriptive cross-sectional study was carried out from April 2022 to December 2023 in Velayat hospital, the only burn center of Guilan province, Iran. Patients with evidence of wound discoloration, purulent drainage, abnormal granulation tissue, abnormal foul odor, increasing temperature, additional breakdown, edema, induration and erythema were tested for fungal infections (11). After 5 days of hospitalization, the bandages were removed and the wounds were washed with saline. The wounds were swabbed and cultured as follows: A sterile cotton swab was moistened with sterile normal saline. This swab was rubbed onto the burn wound surface (11). Swabs were taken from areas which appear deep, areas with discharge or thick scar twice weekly. The swabs were then sent immediately for culture. All specimens were cultured on Sabouraud Dextrose Agar (SDA) with chloramphenicol (SC medium, Merck, Germany). Culture media were surveyed after incubation at 30°C for 48-72 hours. Culture media for those cases with no growth were monitored for 6 weeks. Any growth obtained was further identified by its rate of growth, colony morphology and lactophenol cotton blue mounts. Slide culture was performed as required (12).

Patients were followed to the point of their discharge or death. Patients who had taken any topical or systemic antifungal agents before enrollment were excluded from the study in order to prevention of false-negative results. The medical records of included patients were retrieved and reviewed. Information

was obtained about basic demographic characteristics (age and gender), clinical findings (erythema, unexplained persistent pyrexia, localized pain, discharge from the wound , increased wound size/depth, wound dehiscence, and problems with wound healing), underling disease, burn degree, invasive devices used, duration of hospitalization, the history of antibiotic therapy, and the history of steroid therapy. In this study, criteria such as clinical evaluation, microscopic examination of wound samples (showing budding yeasts and pseudohyphae), and significant growth of pure creamy mucoid colonies on culture media were considered to distinguish between *Candida* colonization and infection of the burn lesion in patients who met the inclusion criteria (8, 13). Yeast isolates were identified based on production chlamydoconidia in cornmeal agar (Becton, France) and colony color on chromogenic CHROMagar *Candida* medium (CHROMagar, Paris, France) (14). Furthermore, for confirmation of identification, all isolates were subjected to PCR and sequencing techniques.

DNA extraction. Fungal genomic DNA was extracted from harvested colonies using glass bead disruption method (15).

PCR conditions and sequencing. PCR amplification for each isolate was performed as described previously (15). To discriminate *Aspergillus* isolates at the species level the Beta tubulin gene of *Aspergillus* species was amplified using the forward (Bt2a: 5'-GGTAACCAAATCGGTGCTGCTTTC-3') and reverse (Bt2b: 5'- ACCCTCAGTGTAGTGACCCTTGGC-3') primers. Also, for identification of other fungal isolates in the species level the universal primers used for fungal amplification were ITS1 (5′TCC GTA GGT GAA CCT GCG G 3′), which hybridizes at the end of 18S rDNA, and ITS4 (5′TCC TCC GCT TAT TGA TAT GC 3), which hybridizes at the beginning of 28SrDNA (Life Technologies, Barcelona, Spain). Positive PCR products were sent for sequencing at Bioneer Advanced Nucleic Acids core facility. Sequences were then separately used to perform individual nucleotide–nucleotide searches using the BLASTn algorithm at the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Fungal identifications were made based on maximum identities ≥ 99% and query coverage ≥ 98% with this method.

Statistical tests. The data analysis was performed by SPSS software (V.20). The study was assessed by using standard Chi-squared and 95% Confidence intervals (CI). Statistically, P value < 0.5 was considered as significant difference or correlation.

RESULTS

In the present study, during a period of 21 months (April 2022 to December 2023), 380 swab specimens were taken from 380 patients with burn lesions. Of those, 101 (26.57%) patients with fungal burn wound infections were diagnosed. The mean age of patients with fungal burn wound infections was 37.93 years (age range of 1 to 82 years). Current research observed that a high correlation existed between the ratio of people over 30 years old and fungal burn wound infections ($p< 0.05$). There were 67.33% (68/101) male patients and 32.67% (33/101) female patients and the results of statistical analysis showed a significant correlation between gender and prevalence of fungal burn wound infections ($p< 0.05$).

In this study, the most positive culture results were observed in patients whose time interval between hospitalization and sampling were greater than or equal to 5 days. Overall, most patients resided in the urban settings (n=91, 90.1%). Also, more than 50% of the patients had an educational level of superior or higher.

Among patients with fungal burn wound infections flame (n=38, 37.63%) was the predominant cause of burns, followed by hot fluid (n=33, 32.67%), electricity (n=15, 14.85%), chemicals (n=10, 9.9%), and hot object (n=5, 4.95%).

The majority of the patients had third-degree burns (n=55, 54.5%), followed by second-degree (n=38, 37.6%) and fourth-degree burns (n=8, 7.9%).

Previous history of ICU admission prior to burn wound infections, presence of central venous catheter before burn wound infections, diabetes mellitus, hypertension, tracheal intubation, steroid use before burn wound infections, heart failure, hypothyroidism, blood disorders, autoimmune diseases, chemotherapy, and viral infections were found in patients with fungal burn wound infections, respectively (Table 1). These predisposing factors were not significantly associated with the occurrence of fungal burn wound infections ($p> 0.05$).

Among 101 positive cases, erythema (n=52, 51.48%), localized pain (n=44, 43.56%), discharge from the wound (n=29, 28.71%), un explained persistent pyrexia (n=21, 20.79%), problem with burn healing (n=7, 6.93%), and wound dehiscence (n=5, 4.95%) were the

Table 1. The detailed information related to 101 patients with fungal burn wound infections

Variable		Patients	Number	Percentage
Age groups (years)		≥30	75	74.26
		< 30	26	25.74
Gender		Female	33	32.67
		Male	68	67.33
The time interval between		<5	40	39.6
hospitalization and sampling (days)		≥ 5	61	60.4
	Residence	Urban	91	90.1
		Rural	10	9.9
	Education	Primary	43	42.58
		Superior	30	29.70
		University	28	27.72
	Degree of burns	First-degree (superficial)	0	0
		Second-degree (partial thickness)	38	37.6
		Third-degree (full thickness)	55	54.5
		Fourth-degree	8	7.9
Underlying conditions and risk factors		ICU admission prior to burn wound infections	35	34.66
		Tracheal intubation	9	8.9
		Presence of central venous catheter before burn wound infections	25	24.75
		Steroid use before burn wound infections	3	2.97
		Diabetes mellitus	17	16.83
		Hypertension	16	15.84
		Heart failure	3	2.97
		Chemotherapy	2	1.98
		Hypothyroidism	3	2.97
		Autoimmune diseases	2	1.98
		Blood disorders	3	2.97
		Viral infections	2	1.98
		History of skin graft procedure	71	70.29
Broad-spectrum antibiotic therapy before burn wound infections			101	100
Cause of burn		Flame	38	37.63
		Hot fluid	33	32.67
		Electricity	15	14.85
		Chemicals	10	9.9
		Hot object	5	4.95
Outcome		Full recovery	2	2
		Partial recovery	94	93
		Discharge by personal satisfaction	3	3
		Death	2	2

clinical symptoms of infected patients, respectively. Statistical analysis showed that there was not a significant relationship between developing fungal burn wound infections and clinical findings ($p > 0.05$).

All patients were under systemic, curative antibiotic therapy for bacterial infection (n=101, 100%). Fluconazole was the only antifungal used for the treatment of fungal infections in the studied burn hospital. A total of 71 patients (70.29%) received skin grafts for their burns. In the current study, 2 out of 101 patients with fungal burn wound infections (2%) died.

The detailed information related to 101 patients with fungal burn wound infections is shown in Table 1.

Mycological and molecular analysis showed that

Candida parapsilosis complex (n=36, 35.64%), and *Pichia kudriavzevii (Candida krusei)* (n=8, 7.92%) represent the most commonly isolated species in the current study (Table 2). The pattern of fungal infections in our cases was single-species infection, which means that only one type of fungal species was isolated from each infected patient and there was no case of mixed fungal infection in the present study. Fig. 1 shows the color of various *Candida* spp. on CHROM agar for identification. Also, Fig. 2 shows chlamydospore production by *C. albicans* on Corn Meal Agar. Corn Meal Agar stimulates sporulation of *C. albicans*, and is useful in suppressing other *Candida* spp. growth.

It should be noted that in the current study, all sequencing results were consistent with macroscopic and microscopic identifications of the isolates.

Table 2. The frequency distribution of fungal elements isolated from 101 patients with fungal burn wound infections in the present study

Isolated fungi	Number	Percentage
Candida parapsilosis complex	36	35.64
Candida krusei	8	7.92
Aspergillus flavus	7	6.93
Candida tropicalis	7	6.93
Curvularia hominis	5	4.95
Rhodotorula mucliginosa	5	4.95
Candida albicans	4	3.96
Candida dubliniensis	3	3.96
Cladosporium cladosporioides	3	3.96
Lecanicillium species	3	3.96
Mucor circinelloides	3	3.96
Penicillium citrinum	3	3.96
Alternaria alternate	2	1.98
Aspergillus niger	2	1.98
Aspergillus sydowi	2	1.98
Candida glabrata	2	1.98
Cladosporium sphaerospermum	2	1.98
Cladosporium tenuissimum	2	1.98
Penicillium aurantiacum	2	1.98

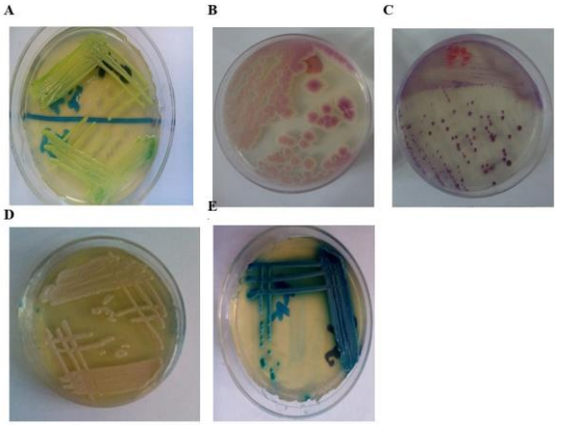

Fig. 1. A) Light green: *C. albicans;* B) Rose pink: *Pichia kudriavzevii (Candida krusei)*; C) Dark violet: *C. glabrata;* D) White to cream: *C. parapsilosis* complex; E) Metallic blue: *C. tropicalis*

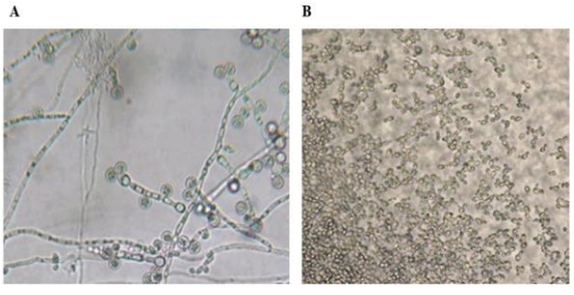

Fig. 2. A) Chlamydospore formation by *C. albicans* on Corn Meal Agar (10X)
B) *Candida* spp. On Corn Meal Agar

DISCUSSION

The results of this survey have further suggested that fungal infections in burns patients should not be underestimated. Despite being routinely cleaned by antiseptic agents, fungal and bacterial colonization in major burns is almost inevitable (16). Recent changes in bacterial flora caused by topical and systemic antibiotics might be a major cause of such a surge in the incidence of fungal infections (5). In addition, lack of clinical suspicion, having clinical presentations similar to bacterial infections, and difficulty in diagnosis are other causes of such increase in the rate of fungal infections (5). Most frequently, fungi come from the environment as burn patients roll on the floor to extinguish flames, use running water to wash chemical and fire burns and use contaminated bandaging left open to the air. Other environmental foci described are air conditioning vents and floor drains (17, 18). Often infection arises from the patient's own flora (19, 20). In the present study, *Candida parapsilosis* complex was identified as the most common cause of fungal burn wound infection in the investigated patients. This finding is in accordance with that reported in a previous study (21). *Candida parapsilosis* is of increasing interest in hospitals, especially in burn centers (22). In addition, *Candida parapsilosis* is commonly found on the hands of hospital workers;

therefore, there is a risk for nosocomial infections transmitted by these professionals (22, 23). After *Candida albicans*, it is the most commonly isolated fungus during burn treatment at hospitals (23, 24). In addition, there have been reports of invasive fungal infections by *Candida parapsilosis* originating from the wound or dressing (21, 25). *Candida parapsilosis* is one of the most common non-albicans *Candida* (NAC) species, and it has emerged mainly due to the indiscriminate use of azoles (26). In addition, this species has been shown to easily adhere to central venous catheters, mechanical ventilation tubes, total parenteral nutrition, and vesical catheters (21).

The incidence rate of fungal burn wound infection in our study was 26.57%. Okuno et al., reported a fungal wound infection incidence rate of 19.44% in burn patients (21). The incidence rate of fungal burn wound infection in our study is lower than that reported in burn tissue biopsy cultures (29%) (25), but is higher than that reported by other authors (12%) (27). These possible explanations for this discrepancy are differences in sampling methods and the time point at which the samples were collected, i.e., immediately after balneotherapy, cleansing, and thorough irrigation with 2% chlorhexidine or saline.

In the current study, the mortality rate was obtained as 2%. However, results of a study obtained by Horvath et al., reported an overall mortality rate of 7.8 in patients with fungal burn wound infection (28). Also, the overall mortality rate in patients with confirmed fungal wound infections in a study conducted by Bahar et al., was 64.3% (29). The mortality rate following fungal infections in the burn patients depends on TBSA, infection locations, and fungal species. Results of studies showed that infection with *Aspergillus* spp. is associated with an especially higher (12-fold) mortality when compared to other mycotic infections found in burn patients. This is most likely a reflection of the angioinvasive tendencies of the mold (30, 31).

In the present study, the time interval between hospitalization and sampling in patients with positive fungal cultures was ≥ 5 days. Based on the literature, prolonged hospitalization predisposes critically ill patients, including burn patients, to secondary fungal infections (32, 7). In accordance with the results of present study, some studies have shown that fungal infection commonly appears after 2-4 weeks of hospitalization (33, 34).

Although this study includes a database of 380 patients, a bigger population could have been used, expanding the time of study, which could reveal other findings that our study fell short of demonstrating. Better differentiation of colonization and infection should have been used in order to demonstrate a stronger correlation between analyzed variables and fungal infection. Using tissue taken from the wound lesion after debridement is better than sampling using swab.

CONCLUSION

In the present study, non-albicans *Candida* species were much high frequent than *C. albicans* with most cases associated with *Candida parapsilosis* complex. Temporal recording of fungal infection diagnosis in the Burn centers would have shown some interesting data regarding fungal infection timing in hospitalized patients.

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