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IDENTIFICATION OF FUNGI IN BURN WOUNDS USING CONVENTIONAL AND VITEK SYSTEM IN DUHOK CITY, IRAQ.

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ABSTRACT:

Background: Fungal burns and wounds infections are frequent but underestimated causing invasive infections with late-onset morbidity and mortality in patients suffering severe perturbed immune systems.

Objectives: This study aimed to investigate fungal infections in clinical specimens by using conventional and Vitek system.

Methods: A total of 123 swabs were obtained from wound and burns patients with different age, gender, burn degrees and nationality that admitted burns and emergency hospital in Duhok city, Iraq, from October 2019 till January 2020. All swabs screened by direct examination, fungal isolation then subjecting the purified colonies to Vitak system 2 to confirm the identification of fungal species.

Results: Out of 123, 55.5% and 44.5% revealed fungal growth in wound and burn swab, respectively with more mono-fungal growth patterns. Male, middle ages group, Iraqi nationality and second-degree burn were more affected. *Cryptococcus laurentii* was the predominant (40%) followed by *Stephanoascus ciferri* (23%), *Aspergillus nigar* (11%) and *Candida albicans* was very low rate (1.1%), while other fungal specie's were recorded in fewer rates. The present study demonstrated that the wound and burn fungal infection cases are relatively high in above mentioned hospitals with a variety of fungal pathogens. Unexpectedly, the *Cryptococcus laurentii* and *Stephanoascus ciferri* were highly predominant.

Conclusions: The study concluded the necessity of using Vitak system for full identification and emphasize on the cleaning of surroundings of patients in the burn and wound units care, reduction of humidity, regular employment of topical and systemic antifungal agents that reduce morbidity and mortality in burn unit in this setting.

KEYWORDS: Burn wound Infections, Fungal infections, Cryptococcus laurentii, Burn Hospital, Vitek system

1. INTRODUCTION

Burn patients are unprotected to a high risk of emerging fungal infections, compared with other hospitalized patients. (Jarvis, 1995) Though frequently underestimated, so opportunistic fungi can causes invasive burn wound infections that ultimately lead to late-onset morbidity and mortality particularly among patients have major burns and/or suffering severe perturbed immune systems. (Church *et al*, 2006). There are numerous predisposing factors play an increasing role in the deterioration of condition namely; the burned total body surface area (TBSA), impaired immune defence, use of broad-spectrum antibiotics that eradicate of the natural bacterial flora and promotion of opportunistic species, increased age, uncontrolled diabetes, and the presence of central venous catheters. (Gore *et al*, 2001; Ballard *et al*, 2008; Luo *et al*, 2011). Despite advances in the use of topical and parenteral antimicrobial therapy, and the practice of early tangential excision, bacterial and fungal infection remains a major problem in the management of burn victims today (De Macedo, 2003).

When a patient undergoes the changes in microbial flora induced by systemic and topical chemotherapy, predisposing of burn wound fungal colonization will increase. Furthermore, the origin of those fungi might be not the gastrointestinal tract but would be complicated by fungemia (De Macedo and Santos, 2005). The chance of fungal colonization of burn wound was found more commonly occurred after third and fourth week post-burn (Bruck *et al*., 1972) Moreover, about 75% of the mortality following burn injuries was related to infections, rather than osmotic shock and hypovolaemia (Colombo *et al*., 2003).

Many studies demonstrated *Candida* spp and other fungal species such as mold *Aspergillus spp* and members of mucorals in burn wounds, such as Al-Hassany, 2017 in Iraq; Al-Aali, 2016 in Saudi Arabia and Dahag *et al* ,2018 in Bahrain. Due to limitations of direct examination in accurate diagnosis of fungi, the other modern multi-function automated machine such Vitek 2 system has been applied. Two studies showed role of Vitek system in full species identification of fungal pathogens isolated from burn wounds (de Macedo and Santos, 2005; Sundaram and Navaneethakrishnan, 2016). Therefore, the authors intend to through insight on the identification of fungal of burn wounds and their prevalence which have crucial importance for expanding the possibilities to develop a reliable therapeutic choice in our area. The purpose of this prospective study is to detect the current fungal profile of burn wounds by using conventional and Vitek system in Burn hospital, Duhok city, Iraq

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2. MATERIALS AND METHODS

2.1 Study setting and population

This is a prospective study conducted in the burn and emergency hospital, Duhok city from October 2019 till January 2020. During this period, a total of 130 patient having acute burn injuries and wounds were included

2.2 Inclusion Criteria

Patients not treated with any antifungal treatment.

2.3 Exclusion Criteria

Patients already treated with any antifungal treatment

2.4 Sample Collection Procedure

The samples were collected from different wound sites of in and outpatients admitted in the burn unit in hospital in Duhok city. Swabs were taken once time from the patients. Briefly, on the day of the patient's admission surface swab was taken, where the tip of the swab was rolled on its side for one full rotation over 4 cm2 of the part of the wound granulation tissue with the most obvious signs of infection and/or inflammation. The swabs were transported within 1 hour to the Microbiology Laboratory for sample processing.
One swab examined after adding 10% KOH solution for direct fungal identification and other swabs for culture on Sabouraud's Dextrose agar (SDA) media supplemented by chloramphenicol (0.05g/L) and gentamicin (0.05g/L) (Difco) and blood agar. The samples were collected under complete aseptic conditions. After incubation for 18-48 h at 25°C, the isolates identified using conventional protocol. Fungal cultures obtained on SDA and on blood agar at 37°C observed daily for one week. The characterization of fungi done by the germ tube test and morphological examination but then subjected to automated method VITEK 2 identification system (bioMérieux Vitek, Inc., MI, US) for more confirmation and specification of isolated fungi at species levels according to (Sundaram and Navaneethakrishnan, 2016).

2.5 Sample Preparation for Vitek system

A 24 hr old culture tested on the instrument, VITEK 2 compact cassette with labelled barcode. Cassette number has defined. The polystyrene tubes were placed in the cassette; 3 mL of sterile Vitek saline take in the polystyrene tubes. The colonies isolated from the plate and suspended in Vitek saline (3 mL) with help of sterile loop. The suspension made uniform thoroughly. The density of the inoculums checked by the Densichek plus. McFarland standard (Vitek 2.20 YST and Vitek 2 YST Card) checked by the Densichek plus provided by the BioMerieux. The cards were then ready for inoculation. All the inoculums in cassette got ready to be loaded into the instrument in the filler section (Sundaram and Navaneethakrishnan, 2016).

3. RESULTS

Out of 123 patients examined, 92 cases (74.8%) of swabs cultures were positive that distributed between 51 (55.5%) and 41 (44.5 %) case from wounds and burns samples, respectively. Most of the included patients were more than 15 year, male, Iraqi nationality, and having second burn degree as in Table (1).

Table 1. Patients description included in this study.

Patient details	No. (%)
Total no. of admissions	123
Total of Positive Fungal culture cases	92 (74.8)
Burn Swab culture positive cases	41 (44.5)
Wound Swab culture positive cases	51 (55.5)
Mean age, (Range)	37(1-90year)
Gender	
Male	70 (56.9)
Female	53 (43.1)
Degree of Burn …Out of 41 positive cases	
1st	7 (17)
2nd	30 (73)
3rd	4 (10)
Prevalent burn cause in different age groups	
>15 years	8 (19.5)
<15 years	33(80.5)
Patient residency	
Iraqi	113 (91.9)
Syrian immigrants	10 (8.1)

Table 2. Total number of positive wound and Burn swabs

Total no. of positive burn swabs	41
Mono-fungal growth	32 (77%)
Poly-fungal growth	9 (23 %)
Total no. of positive wound swabs	37
Mono-fungal growth	45 (88 %)
Poly- fungal growth	6 (12%)

On another hand, most of the burn and wound swab cultures positive were more frequent with mono-growth fungal species (i.e. yielding single species) than ploy-growth as in Table (2).
According to protocol of direct examination of each swab sample and then later subjecting the culture to Vitak machine 2, a diversity of 10 fungal species' have been identified from both burn and wound swab cultures as in Table (3). It has been found generally that wound swabs sampling performed yielded more kind of fungal species than burn one and the fungal species *Cryptococcus laurentii* was the predominant species accounted (40%) followed by *Stephanoascus ciferri* (23%) and 11% was *Aspergillus nigar*, while other fungal specie's were recorded in fewer rates.
Generally, most fungal infections were seen among patients having burns percentage (20-35%) and remarkably was caused by *Cryptococcus laurentii* while; *Stephanoascus ciferri* was the exclusive pathogen detected in patients having 55% burn percentage, as in Table (4).

Fungal spp	Within Burn Swab No. (%)	Within Wound Swab No. (%)	Total (%)
Cryptococcus laurentii	17	20	37 (40)
Stephanoascus ciferri	8	13	21 (23)
Aspergillus niger	5	5	10 (11)
Aspergillus flavus	4	3	7 (7.5)
Trichosporon mucoides	2	7	9 (9.8)
Penicillium sp	2	2	4 (4.3)
Candida albicans	0	1	1 (1.1)
Rhodotorella sp	1	0	1 (1.1)
Chladosporium	1	0	1 (1.1)
Dermatophyton spp	1	0	1 (1.1)

Table 3. Frequency of fungal isolates in wound and Burn swabs.

4. DISCUSSION

Infections of burns with fungal agents are not surprising in any setting area and community because many predisposing factors will prone to infection. For this reason, the current study performed to look for diverse of fungal etiologies of burns after the admission of patients and fully identification of isolates using Vitak system2. In this study, 41/123 (44.5%) cases of burns showed positive growth of various numbers of 10 different fungal isolates. Other studies, in Iraq reported that 90% of fungal burn infections were attributed to 13 different fungal isolates (Al-Hassani, 2007). The current study revealed that the incidence of burns fungal infections were very less than that reported by (Santucci *et al*, 2003; Ballard *et al*, 2007). Many factors could contribute to acquiring fungal infections exogenously in care units' appendages such as walls, beds, mattresses, and dressing instruments that may be sources of funguses (Mousa,2016) and endogenously for instance, presence of coagulated proteins, the absence of blood-borne immune factors, and the avascularity of the burn wound (De Macedo *et al*., 2005).

Table 4. Correlation of frequency between isolated fungi and percentage of burn

Fungal Isolates	No. of fungal species among burns of various percentage %							
	10	15	20	25	30	35	40	55
Cryptococcus laurentii	1	1	2	4	1	6	1	
Stephanoascus ciferri		2	1	3				5
Aspergillus niger			1	1		2		
Aspergillus flavus					2	2		
Trichosporon mucoides		1				1		
Penicillium sp					1			
Candida albicans								
Rhodotorella sp			1					
Chladosporium				1				
Dermatophyton spp	1							
Total: (41)	2	4	5	9	4	11	1	5

The prevalence of infections details that occurred in both burn and wound swabs in the current study were mainly mono-fungal growth than ploy-fungal growths. Identification of molds and yeasts species might be time-consuming because specific cultures will be needed to recover these organisms are not within routine work employed for all cases. So Vitak system may overcome this diagnostic obstacle especially for a high level of suspicion of fungal cultures in suspected cases. In the current study, based on findings of the Vitek system, 10 different fungal isolates were identified to species level. *Cryptococcus laurentii* was the predominant species accounted (40%) followed by *Stephanoascus ciferri* (23%). In contrast Al-Hassani, 2007 found that *Aspergillus niger* (14.4) was predominant among burns infections compared with results we obtained that the *Aspergillus niger* was the third pathogen which accounted 11% of cases. Recent study 2019 in China, detected this fungal species in pulmonary cryptococcosis among immunocompromised patients (Zhang *et al.*, 2019). Several years, *Cryptococcus laurentii* believed to be saprophytic and non-pathogenic to humans, but this concept has recently been challenged with increasing incidence of opportunistic infections in old and immunocompromised patients namely fungemia and pulmonary cases (Khawcharoenporn *et al.*, 2007; Banerjee *et al.*, 2013). The high rate of *Cryptococcus laurentii* in present study was unexpected because it is uncommon pathogen and scarce data in our setting. Moreover, Unnoticeable expansion beyond its normal environmental niche became recognize it as human pathogen in burns and wounds infections in Kurdistan hospitals that need re-schedule of infection control programs. Many studies confirmed that water, soil, milk, cheese droppings and cloacal samples of feral pigeons (city pigeons) may be responsible for both deep-seated infections such fungemia and meningitis and superficial infections such as keratitis in immunocompromised persons (Mattsson *et al.*,1999; Banerjee *et al.*, 2013; Zhang *et al.*, 2019).

In the current study, *Stephanoascus ciferri* accounted (23%) of cases with relatively higher among wound swab than burns one. It was occurred mainly in severe burns infection and burn percentage (55%). This fungal species did not record in other studies in Iraq (Al-Hassani, 2007), In Mexico, this unusual pathogen was isolated from the blood of a patient with Crohn's disease (Villanueva-Lozano *et al*., 2016). Also, *S. ciferrii* was isolated from blood of an AML (acute myeloid leukemia) patient, and from wound swab, aural discharge and nail sample especially from immunodeficiency patients (Soki *et al*., 2010; Capoor *et al*., 2015).

Regarding *Candida albicans* in the current study, only one case was found in wound swab, which disagree with that reported by Al-Hassani, 2007, as 6.6% of *C albicans* in burn swab. Moreover, other studies considered *C albicans* and other non *C albicans* as the most frequent pathogenic species of burns infections as 50%, 66.5% and 16.7 % by (De Macedo *et al*, 2005; AL-Aali, 2016; Dahag *et al*., 2018), respectively. Lost of keratin may reduce to a certain extent the growth of fungal species in burns compared with wound. Providing adequate air-conditioning that reduces humidity in burn care units will eventually assist in reduction of fungal growth and spread. Furthermore, conservative or well-adjusted usage of antibiotics for burned patients is also diminishing fungal and yeast infection incidence.

5. CONCLUSION

This study comes to the conclusion that the wound and burn fungal infection cases are relatively high in above-mentioned Kurdistan hospital with variability of fungal burn infections. The growth rate of *Candida albicans* was fairly low in burn and wound infection compared with predominant of *Cryptococcus laurentii* and *Stephanoascus ciferri*. This finding is important for highlighting using the modern technique such as Vitak system for accurate identification of fungal isolates. Also, emphasis on the hygiene in the burn and wound units care in addition to build more efficacious treatment regimens in particular topical and systemic antifungal agents that reduce the contamination in burn and wound unit.

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REFERENCES

AL-Aali , K.Y. (2016). Microbial Profile of Burn Wound Infections in Burn Patients, Taif, Saudi Arabia. *Arch Clin Microbiol*, 7-2.

Al-Hassani , K. A. (2007). Fungal study of burns iinjury iin patiients of All-Qadiissiiyiia rovince. *Al Qadissia pure science journal*, 1, 134-141.

Ballard, J., Edelman, L., Saffle, J. (2008). Positive fungal cultures in burn patients: a multicenter review. *J Burn Care Res*, 29, 213-21.

Ballard, J., Edelman, L., Saffle, J., Sheridan, R., Kagan, R., et al. (2008). Positive fungal cultures in burn patients: a multicenter review. *J Burn Care Res*, 29, 213-221.

Banerjee, P., Haider, M., Trehan, V., Mishra, B., Thakur, A., Dogra, V., et al. (2013). *Cryptococcus laurentii Fungemia*. *Indian J Med Microbiol*, 31,75-7.

Bruck, H.M., Nash, G. Stein., J.M., Lindberg., R.B. (1972). Studies on the occurrence and significance of yeasts and fungi in the burn wound. *Ann Surg*, 176,108-110.

Capoor, M. R., Gupta, D. K., Verma, P. K, Sachdeva, H. C. (2015). Rare yeasts causing fungemia in immunocompromised and haematology patients: Case series from Delhi. *Indian J Med Microbiol*, 33, 576-9.

Churc, H. D., Elsayed, S., Reid, O. (2006). Burn wound infections. *Clin Microbiol Rev*, 19, 403-34.

Colombo, A.L., Guimarães, T. (2003). Epidemiology and hematogenous infection of *Candida* spp. *Rev Soc Bras Med Trop*, 36, 599-607.

Dahag, M.A., Louri, N.A., Dey, N., Philip, S.S. (2018). Pattern of the Burn Wounds Infections in Bahrain Defence Force Military Hospital. *Ann Burns and Trauma*, 2(1), 1007.

De Macedo, J.L., Rosa, S.C., Castro, C (2003). Sepsis in burned patients. *Rev Bras Med Trop*, 36:, 647-652.

De Macedo, J.L., Santos, J.B. (2005). Bacterial and fungal colonization of burn wounds. *Mem Inst Oswaldo Cruz*, 100(5), 535-539.

Gore, D.C., Chinkes, D., Heggers, J. (2001). Association of hyperglycemia with increased mortality after severe burn injury. *J Trauma*, 51, 540-4.

Jarvis, W.R. (1995). Epidemiology of nosocomial fungal infections, with emphasis on candida species. *Clin Infect Dis*, 20, 1526-30.

Khawcharoenporn, T., Apisarnthanarak, A., Mundy, L.M. (2007). Non-Neoformans cryptococcal infections: a systematic review. *Infection*, 35(2), 51–8.

Luo, G., Peng, Y., Yuan, Z. (2011). Yeast from burn patients at a major burn center of China. Burns, 37, 299-303.

Mattsson, R., Haemig, P.D., Olsen, B. (1999). Feral pigeons as carriers of *Cryptococcus laurentii*, *Cryptococcus uniguttulatus* and *Debaryomyces hansenii*. *Med Mycol*, 37, 3679.

Mousa, H.A . (2016). Fungal Burn Wound Infection. *Fungal Genom Biol*, 6, 121.

Santucci, S.G., Gobara, S., Santos, C.R., Fontana, C., Levin, A.S . (2003). Infections in a burn intensive care unit: experience of seven years. *J Hosp Infect*, 53:6-13.

Soki, H., Nagase, Y., Yamazaki, K., Oda, T., Kikuchi, K. (2010). Isolation of the yeast-like fungus *Stephanoascus ciferrii* by culturing the aural discharge of a patient with intractable otitis media. Case report. *Kansenshogaku Zasshi* , 84,210-2.

Sundaram, M., Navaneethakrishnan, R.M. (2016). Evaluation of Vitek 2 system for clinical identification of candida species and their antifungal susceptibility test. *J Evolution Med Dent* Sci, 5(47), 2948-2951

Villanueva-Lozano, H., Treviño-Rangel, R., Hernández-Balboa, C., Gloria, M., Martínez-Reséndez, M., et al. (2016). An unusual case of *Candida ciferrii* fungemia in an immunocompromised patient with Crohn's and *Mycobacterium bovis* disease. *J Infect Dev Ctries*, 10(10),1156-1158.

Zhang, Y., Cooper, B., Gui, X., Sherer, R., Cao., Q. (2019). Clinical diversity of invasive cryptococcosis in AIDS patients from central China: report of two cases with review of literature. *BMC Infectious Diseases* , 19:2-8.