Frequency and Antifungal Susceptibility Pattern of *Candida* Species Causing Candidemia in Bone Marrow Transplant Unit

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ABSTRACT

To determine the frequency and antifungal susceptibility pattern of *Candida* species causing candidemia or blood stream infections (BSI) in immunocompromised patients of a bone marrow transplant unit. The study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi in collaboration with Armed Forces Bone Marrow Transplant Center (AFBMTTC), Rawalpindi, from 1st June 2019 to 31st December 2019. A total 256 paired blood culture samples from patients of all ages, irrespective of gender were collected during a period of seven months. The samples were processed as recommended Clinical & Laboratory Standard Institute (CLSI) guidelines. Antifungal susceptibility by break point MICs was performed through VITEK® 2 system (version: 08.01) for Fluconazole, Itraconazole and Amphotericin B. Of the total 256 blood cultures, 46 (17.97%) were positive for *Candida* species. The susceptibility of *C. albicans* was 100% to Amphotericin B, 90% to Fluconazole and 10% to Itraconazole, while *C. tropicalis* showed sensitivity of 92%, 88% and 0% against Amphotericin B, Fluconazole and Itraconazole, respectively. *C. parapsilosis* was found sensitive to Amphotericin B while resistance to Fluconazole and Itraconazole. Frequency of candidemia among immunocompromised patients is 17.9%. *C. tropicalis* and *C. albicans* are the two most common *Candida* species involved in blood stream infections in our setup. Fluconazole and Amphotericin B, both were found susceptible and can be used as empirical therapy.

Keywords: Anti-fungal Susceptibility, Blood Stream Infections. Candidemia, Immunocompromised Patients, *Candida albicans*

Article History:

Received: 22 November 2020

Revised: 15 Jan 2021

Accepted: 18 Jan 2021

Published: 20 Jan 2021

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INTRODUCTION

Candida species, common commensals of the skin and mucosa of gastrointestinal tract are opportunistic pathogens, causing diseases ranging from superficial infections like oral thrush to systemic infections (candidemia)¹. The last decade has seen a rise in invasive *Candida* infections due to increase

in immunocompromised population, the vast use of invasive devices, prolonged hospitalization in the intensive care units (ICU) and injudicious use of broad spectrum antimicrobial agents.² The incidence has risen specially in specific setting where more aggressive therapy practices

(chemotherapy, invasive devices etc.) are being used with some contribution from the use of immunosuppressive modulators for management of autoimmune disorders^{2, 3}. In United states it has been ranked as the fourth most prevalent pathogen of nosocomial blood stream infections (BSI)³.

Among *Candida* species, *Candida albicans* represents the principal pathogen, accounting for 90% of infections, however due to improved diagnostic methodologies, non-*Candida albicans* *Candida* species are being isolated with increasing frequency, especially in immunocompromised population. Moreover, rapid development of resistance among these species is concerning⁴.

Candidemia is associated with significant mortality. Its incidence and distribution vary based on geographical locations and patient population^{2, 5}. Rapid and timely therapeutic intervention is required for a favorable prognosis^{6, 7}. Therefore, it is important to study the frequency and antifungal susceptibility patterns of *Candida* blood stream infections since data from Pakistan is lacking. Empirical treatment protocols must be based on local susceptibility data. This study was carried out to determine the frequency, pattern and antifungal susceptibility of *Candida* species causing blood stream infections in immunocompromised patients from a bone marrow transplant unit at Rawalpindi.

METHODOLOGY

This Cross-sectional descriptive study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), in collaboration with Armed Forces Bone Marrow transplant center (AFBMT) Rawalpindi, Pakistan (a 54 bedded unit) from June 2019 to December 2019. The study was commenced after taking approval from the Institutional ethical committee (IEC). All non-repetitive blood culture samples received from AFBMT were included in the study through non-probability consecutive sampling. Clinical specimens were given specific identification numbers to maintain anonymity. The patient population for the study included bone marrow transplant recipients, and patients with hematological malignancies admitted in AFBMT, without age and gender discrimination. An episode of candidemia was defined as paired blood culture samples yielding growth of *Candida* species.

Paired blood culture samples were aseptically collected from the patients clinically suspected of sepsis. Suspected sepsis was defined by body temperature of <36 or $>38^{\circ}$ centigrade and/or hypotension (Systolic blood pressure <90 mmHg)^{6,7}.

The samples were incubated in BACTEC (Becton Dickinson, USA) and BacT/Alert 3D (biomeriux, France) automated blood culture systems. Signal positive samples were subcultures on 5% sheep blood agar, MacConkey and Sabouraud dextrose agar (Oxoid, UK) plates. *Candida* species were identified by Gram stain, germ tube test, BBL CHROMagar *Candida* (Becton Dickinson, USA) and VITEK® 2.0 systems (version: 08.01). Antifungal susceptibility by break point MICs was performed through VITEK® 2.0 systems (version: 08.01) for Fluconazole, Itraconazole and Amphotericin B. CLSI guidelines were used for all proceedings.

Compilations and statistical analysis of data was performed using IBM SPSS statistics ver.23.00. Descriptive statistics including frequencies and percentages were calculated for *Candida* species positive blood cultures and resistance to the tested antifungals agents.

RESULTS

Out of the total 256 blood cultures included in the study, 46 (17.97%) were detected positive for *Candida* species. Among positive blood cultures, *Candida* species were more frequently isolated from female patients, 26 (56.52%) as compared to males 20 (43.48%) with male to female ratio of 1:1.3.

Frequency of different *Candida* species is shown in figure 1. Overall, *C. albicans* were 20 (43.48%), *Candida tropicalis* 25(54.35%) and *Candida parapsilosis* 1(2.17%). The susceptibility pattern of the three *Candida* species is given in figure 2. *C. albicans* was found 100% sensitive to Amphotericin B, 90% to Fluconazole, and 10% to itraconazole while *C. tropicalis* showed sensitivity of 92%, 88% and 0% against Amphotericin B, Fluconazole and Itraconazole respectively.

DISCUSSION

Candida species are ranked fifth amongst hospital-acquired pathogens and fourth amongst BSI pathogens. Underlying malignancies, immunosuppressive diseases, hematopoietic stem cell or solid organ transplantation, the use of broad-

spectrum antibiotics or corticosteroids, invasive interventions, aggressive chemotherapy, parenteral alimentation, and internal prosthetic devices, all

contribute to the increased risk of invasive candidiasis. These infections contribute to high mortality in hospitalized patients^{2,5,6}.

Fig 1: Frequency of different *Candida* species isolated from blood cultures of immunocompromised patients

Fig 2: Susceptibility pattern of *Candida* species to antifungal drugs

A total of 17.97% blood cultures from immunocompromised patients of bone marrow transplant unit were positive for *Candida* in our

study. A study by Newishy *et al.*, reported 5.5% blood cultures positive for *Candida* species from immunocompromised patients, while it was 6% as

reported in a study from Pakistan. Frequency of candidemia in cancer patients was 1.6% in a study from Lahore, Pakistan⁸⁻¹⁰.

In our study, *C. tropicalis* (54.34%) was found most frequent *Candida* species causing Candidemia in immunocompromised patients, followed by *C. albicans* (43.47%) and *C. parapsilosis*. In a study conducted by Valentina Cataldi et al. (2017) *Candida* species were isolated from different body sites and *C. albicans* (50%) was reported as the most prevalent species, followed by *C. glabrata* (40%) and *C. parapsilosis* (10%)¹. Another study from China has also reported *C. albicans* as the most frequent species causing invasive infections, however non-albicans species were more common in patients with hematopoietic stem cell transplant patients, as in our study². Another study in 2014 by Daichii et al. reported *C. albicans* as the most frequently isolated *Candida* species from blood cultures, 70 cases (43%), followed by *C. parapsilosis* with 36 cases (22%), *C. glabrata* with 25 cases (15%) and *C. tropicalis* and *C. Krusei* with 11 (7%) and 10 (6%) cases, respectively¹¹. Other studies also report similar findings of *C. albicans* being the common cause of candidemia in immunocompromised population as well^{12, 13}. Collectively the non-albicans species have now become a significant cause of candidemia^{2, 5}. *C. tropicalis* being the most frequently isolated *Candida* species is a contrasting finding in our study. This finding has been previously highlighted in other studies from Pakistan, that also reported *C. tropicalis* as the most common species in invasive candidiasis (51.5% and 32.5%, respectively)¹⁴. Another study from Pakistan has also reported a predominance of non-albicans *Candida* as a cause of candidemia, but in contrast to our study *C. parapsilosis* was found to be the most frequent.

Our study showed good susceptibility of *C. albicans* (43.4%) to Amphotericin B (100%) and fluconazole (90%) a finding consistent with results of other studies². *C. tropicalis* also showed good susceptibility to these anti-fungal agents. In contrast, the one isolate of *C. parapsilosis* was sensitive to Amphotericin B while resistant to azoles. All three *Candida* species isolated from BSIs in this study had poor susceptibility to Itraconazole. Good susceptibilities of *C. albicans* and *C. tropicalis* have also been reported in other studies^{2, 6}. A study from China has reported 15.7% resistance to Fluconazole in contrast to the findings of the present study, while

resistance to Amphotericin B was 2.4% for *C. albicans*¹³. Farooqi et al., in their study also reported good susceptibility of *Candida* species to Amphotericin B and Fluconazole, again stressing their reliability as empirical treatment¹⁴.

CONCLUSION

Frequency of candidemia among immunocompromised patients is high (17.9%). *C. tropicalis* and *C. albicans* are two most important and common *Candida* species involved in blood stream infections in our setup. Fluconazole and Amphotericin B both were found susceptible and are reliable empirical treatment options. A need for constant surveillance of the susceptibility patterns is important to timely identify emergence of resistance.

CONFLICT OF INTEREST

None

FUNDING SOURCE

None

AUTHOR'S CONTRIBUTIONS

WH: Gathered references based upon inclusion and exclusion criteria, Methodology write up

SB: Bench work in microbiology laboratory

IAM: Supervising entire manuscript for technicalities, Discussion writing

Al: Supervising the bench work in microbiology laboratory

UK: Introduction write up and result summarization.

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