ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 05 | May - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ADHERENCE OF NON ALBICANS CANDIDA SPECIES TO HUMAN BUCCAL EPITHELIAL CELLS: A POSSIBLE MECHANISM FOR PATHOGENICITY OF MUCOCUTANEOUS INFECTIONS.

Microbiology

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ABSTRACT

Background: Mucocutaneous candidiasis is the commonest clinical manifestation caused by Candida species. Predominance of non albicans Candida (NAC) species over C. albicans is recently the very fact. Among various virulence traits of Candida spp., adhesion play a key role in pathogenesis. In this study, adherence potential of NAC spp. isolated from mucocutaneous infections was evaluated. **Method:** NAC spp. isolated from mucocutaneous infections like vulvovaginal candidiasis (VVC) and oropharyngeal candidiasis were identified upto species level and the property of adhesion was studied by using human buccal epithelial cells (HBEC). **Results:** A predominance of NAC spp., over C. albicans was noted as out of 147 isolates, 128 (87.1%) were NAC spp., whereas 19 (12.9%) were C. albicans. C. tropicalis (50%) was the predominant NAC spp. The rate of HBEC adherence in NAC spp. isolated from VVC was significantly high compared to isolates from OPC. Among NAC spp., the rate of HBEC adherence was high in C. tropicalis. **Conclusion:** NAC spp., is the predominant cause of mucocutaneous Candida infection like VVC and OPC. Treatment failure due to antifungal resistance is the biggest challenging while tackling the infections due to NAC spp. Understanding the virulence trait like adherence would help to unravel the complexity of pathogenesis of NAC spp.

KEYWORDS

Adherence, oropharyngeal candidiasis, pathogenicity, virulence factors, vulvovaginal candidiasis.

INTRODUCTION.

In today's world, the mycotic pathogens in general and Candida in particular have become an imminent threat to mankind. Candida, a fungal opportunist is capable of causing a wide spectrum of clinical manifestations that ranges from mucocutaneous overgrowth to disseminated infections.[1]

Although a hitherto of clinical manifestations once rarely attributed to Candida spp., are increasingly recognized in recent years, in routine clinical practice, mucocutaneous infections are more commonly encountered.[2] Vulvovaginal candidiasis (VVC) and oropharyngeal candidiasis (OPC) are most common manifestations of mucocutaneous Candida infections.[2,3]

Several virulence traits are identified for Candida spp. These include exoenzyme activity, hemolysin production, adherence to various biotic and abiotic substances, and biofilm formation.[4] Among these virulence factors, adhesion is the first and most crucial step in the pathogenesis of Candida infection as it facilitates the attachment to the host's tissues, initiates colonization and biofilm production, which remain the cornerstone for both establishment and persistence of the infection.[5]

Recent pertinent studies have documented the predominance of non albicans Candida (NAC) species over C. albicans in various types of Candida infections including mucocutaneous candidiasis.[6] Although adhesion is well studied in C. albicans, it is still underexplored in NAC spp. Therefore, the present study was performed with an aim to determine the adherence capacity of NAC spp., isolated from mucocutaneous candidiasis to human buccal epithelial cells (HBEC).

MATERIALAND METHODS.

I. Study Design, Population And Setting

The present study was conducted in the Department of Microbiology, Dr. Balasaheb Vikhe Patil Rural Medical College and Pravara Rural Hospital for a period of 3 years (January 2019 to December 2021). The study included the NAC spp. isolated from various mucocutaneous Candida infections like VVC and OPC.

II. Identification Of Candida Species

The identification of Candida isolates was done by phenotypic tests (germ tube test, colony color and morphology on Hichrom Candida agar) and biochemical tests (sugar assimilation).[7] The isolates that were unidentified by conventional techniques (phenotypic and biochemical tests) were identified by automated technique using VITEK 2 system.

III. Adherence of NAC spp. to HBEC

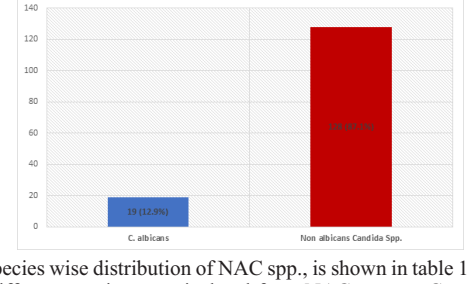
The adherence of NAC spp. isolated from mucocutaneous candidiasis to HBEC by the as per the method described by Al Abied et al. (2004).[8] Buccal epithelial cells (BEC) were collected by gently rubbing the cheek mucosa of healthy laboratory technicians (no signs or symptoms of oropharyngeal candidiasis or other oral lesions and not receiving any antibiotics at the time of study) with a sterile cotton swab after obtaining prior consent. As fresh BECs for assay were used, they were collected on the same day when assay was conducted. BECs were washed thrice by phosphate buffered saline (PBS) and harvested by centrifugation.

Equal volumes (1ml) of BEC (1x105 cells/ml) and yeast suspension (1x107 cells/ml) were mixed and incubated at 37^{0}C for 2 h in a shaking water bath at 40 rpm. The mixture was filtered through a 20 µm filter for removing the non-adherent yeast cells. The BECs on the filter were then washed with 5 ml of PBS and finally suspended in 5 ml of PBS.

A drop of this suspension was placed on glass slide and allowed to dry. After air drying, the smear was fixed by methanol, Gram stained and observed under microscope. The rate of adherence was determined by counting the mean number of yeast cells adhering to 100 BECs.

RESULTS.

During the study period a total of 147 Candida spp. were isolated from mucocutaneous infections. Out of these 147 isolates, 121 (82.3%) were isolated from cases of VVC whereas 26 (17.7%) were isolated from patients with OPC. A predominance of NAC spp., over C. albicans was noted as out of 147 isolates, 128 (87.1%) were NAC spp., whereas 19 (12.9%) were C. albicans (Figure 1).



The species wise distribution of NAC spp., is shown in table 1. A total of 6 different species were isolated from NAC group. C. tropicalis

(50.7%) was the predominant NAC spp. C. krusei was isolated from 22 (17.2%) cases whereas isolation of C. glabrata (11.7%) was noted in 15 (11.7%) cases of mucocutaneous candidiasis.

Table 1. Species wise distribution of NAC isolates.

Non albicans Candida spp.	Vulvovaginal candidiasis (%)	Oropharyngeal candidiasis (%)	No. (%)
C. tropicalis	56 (52.8)	09 (40.9)	65 (50.7)
C. krusei	17 (16.1)	05 (22.7)	22 (17.2)
C. glabrata	10 (9.4)	05 (22.7)	15 (11.7)
C. parapsilosis complex	11 (10.4)	-	11 (8.6)
C. gulliermondii	05 (4.7)	03 (13.6)	08 (6.3)
C. kefyr	07 (6.6)	-	07 (5.5)
Total	**106**	**22**	**128**

A total of 121 (94.5%) NAC spp., isolated from mucocutaneous candidiasis showed ability to adhere to HBEC. In case of VVC, a total of 104 (98.1%) NAC spp., out of 106 showed adherence to HBEC whereas HBEC adherence was seen in 17 (77.3%) isolates from OPC.

The overall mean (± SD) of rate of HBEC adherence in NAC spp. isolated from mucocutaneous candidiasis was 188.25 (±89.8)/100 BEC (range 87-221/100 BEC). The mean (± SD) HBEC adherence rate in NAC spp. isolated from VVC was 181.78 (±72.4)/100 BEC (range 110-215/100 BEC) whereas it was 141.23 (±56.8)/100 BEC (range 81-116/100BEC) in NAC spp. isolated from OPC. The rate of HBEC adherence in NAC spp. isolated from VVC was significantly high compared to isolates from OPC (Mann Whitney Test, P value <0.05*).

HBEC adherence in different NAC spp. is shown in table 2. The rate of HBEC adherence in C. tropicalis isolated from both the OPC and the VVC was significantly high compared to other isolates (Mann Whitney Test, P value <0.05*).

Table 2. HBEC adherence in NAC spp. isolated from mucocutaneous candidiasis.

Non albicans Candida spp.	Vulvovaginal candidiasis			Oropharyngeal candidiasis		
	Total	Adherence (%)	Mean (±SD)	Total	Adherence (%)	Mean (±SD)
C. tropicalis	56	56 (100)	218.23 (±64.6)	09	09 (100)	164.42 (±28.2)
C. krusei	17	17 (100)	132.31 (±55.2)	05	04 (80)	111.32 (±55.4)
C. glabrata	10	10 (100)	117.21(± 41.6)	05	02 (40)	82.21 (±16.2)
C. parapsilosis complex	11	11 (100)	123.65 (±43.2)	-	-	-
C. gulliermondii	05	05 (100)	113.54 (±36.7)	03	02 (33.4)	61.12 (±15.6)
C. kefyr	07	05 (71.4)	94.11 (±38.1)	-	-	-
Total	**106**	**104**	**181.78 (±72.4)**	**22**	**17**	**141.23 (±56.8)**

DISCUSSION.

A varied clinical spectrum of disease and a growing number of patients vulnerable to infection, compounded by the emergence of treatment resistant species makes candidiasis a certainly a worrisome malady.[9] Mucocutaneous candidiasis, an otherwise milder form of the infection that can be cured with practice of basic hygiene and application of topical antifungals may pose as a significant problem in debilitated patients and in infections caused by drug resistant strains.[10]

As noted in this study, it is evident that the incidence of OPC is decreased, as only 26 (17.7%) out of a total of 147 Candida spp. were isolated from the oropharyngeal swabs. Although the incidence of OPC is declined, it still remains a common fungal infection in patients with weakened immune defenses. It has high-rate relapse (20 to 50%) with relapse occurring within 2 weeks of treatment cessation.[11]

A total of 82.3% of Candida isolates were from cases of VVC. VVC, a common fungal infection of lower reproductive tract, occurs in approximately 75% of all females at least once in their lifetime. VVC is a multifactorial infection, predisposed by several conditions like antimicrobial drug therapy, sexual activity, high-oestrogen containing oral contraceptives, pregnancy, use of sodium glucose cotransporter 2 (SGLT2) inhibitors and uncontrolled diabetes mellitus.[12] Unlike other Candida infection, VVC usually manifests in otherwise healthy and immunocompetent women. Although non-fatal, the global epidemiology of VVC is high and has catastrophic on quality of life. Therefore, it is highly necessary to understand the factors (host's and fungal) that drive the pathogenesis of the disease.[13]

As clued by findings of recent studies, predominance of NAC spp., was noted. In this study, 87.1% of isolates belonged NAC spp. Reasons cited for emergence of NAC spp., is rampant use of azoles for treating and preventing fungal infections along with use of over-the-counter antifungal ointments.[13] However, the role of advancement in diagnostic modalities for Candida including the use of chromogenic media, commercial identification system and molecular methods can't be overlooked.[14] C. tropicalis, C. krusei (currently Pichia kudriavzevii), C. glabarta (currently Nakaseomyces glabratus) and C. parapsilosis complex were the common NAC spp.

For studying the pathogenesis of a particular infection, it is very essential to use a carefully combed in vitro method that perfectly fits the jigsaw of the process that occurs in vivo. In this study, adherence to NAC spp., isolated from mucocutaneous candidiasis adherence to HBEC was used study the property of adhesion, which is the main and the most crucial stage of pathogenesis. Technique of adherence to HBEC although simple and cost-effective can perfectly nuance the process of adhesion in Candida spp.[15]

In this study, the rate of HBEC adherence in NAC spp. isolated from OPC was significantly high compared to isolates from VVC. This fact explains the finding that although NAC spp., isolated from cases of OPC had less adhesion capacity, the Candida spp, with less adherence potential can adhere to buccal epithelium and initiate the process of infection in patients with compromised immune status.

Among NAC spp., the rate of HBEC adherence was high in C. tropicalis isolated from both the OPC and the VVC. C. tropicalis is reported to be the most pathogenic among NAC spp.[16] It is reported to produce virulence factors similar to that C. albicans whereas few studies have reported C. tropicalis to produce more aggressive virulence factors than C. albicans.[16]

CONCLUSION.

Emerging fungal infections in general and treatment-resistant candidiasis in particular is one among the current global challenges. A growing number of patients are reported to be both colonized and infected with non albicans Candida (NAC) species which are difficult to treat. Study of virulence factors in these conditionally pathogenic yeasts is very important to extract important insights for understanding the pathogenesis. From our study it can be concluded that NAC spp. has potential of adhesion to host cells and this property aid NAC spp., to initiate the process of infection followed by its dissemination.

Conflict Of Interest: nil

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