Orignal Article

Virulence Factors Attributed to Pathogenicity of Non Albicans Candida Species Isolated From Human Immunodeficiency Virus Infected Patients with Oropharyngeal Candidiasis

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ABSTRACT

Background: Oropharyngeal candidiasis (OPC) is the most common opportunistic mycoses among HIV infected patients. Although Candida albicans is the most common aetiological agent of OPC, in recent years non albicans Candida (NAC) species have become increasingly prominent pathogens. As compared to C. albicans, NAC spp. often demonstrates low susceptibility to commonly used antifungal drugs.

Aim: The present study was conducted with an aim to determine the expression of virulence factors of NAC spp. isolated from HIV infected patients with OPC.

Methods: A total of 123 NAC spp. isolated from HIV infected patients with OPC were included in the study. The virulence factors studied were adherence to buccal epithelial cell, exoenzymatic activity and haemolytic activities.

Results: C. glabrata and C. tropicalis were predominant isolates from NAC spp. ABEC was more in C. dubliniensis isolates. As compared to other NAC spp. C. glabrata demonstrated low ABEC. High phospholipase activity was noted in C. tropicalis followed by C. kefyr. Proteinase activity was high in C. dubliniensis followed by C. tropicalis. Haemolysin production was high in C. tropicalis followed by C. kefyr isolates.

Conclusion: NAC spp. once overlooked as mere contaminants or non pathogenic commensals have emerged as potent pathogens. These isolates are capable of producing virulence factors once attributed only to C. albicans. Knowledge of these virulence factors is important for understanding the pathogenesis of candidiasis and will help to explore new antimycotic drug targets for improved therapeutic regimens.

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Introduction

Oropharyngeal candidiasis (OPC) is the most common opportunistic mycoses among HIV infected patients. [1] At many times it may be the first visible sign of HIV infection and the patient's only chief complaint. [2] OPC develops in 80-90% of HIV infected patients at some point during the progression of their disease. [1]

Although Candida albicans is the most common aetiological agent of OPC, in recent years non albicans Candida (NAC) spp; such as C. tropicalis, C. glabrata and C. krusei have become increasingly prominent pathogens.[1] As compared to C. albicans, NAC spp. often demonstrates low susceptibility to commonly used antifungal drugs. [2]

Candida spp. is often a minor component of commensal oral flora. [3] The transition of Candida spp. from a commensal to a potent pathogen is contributed by several factors including host predisposing factors and virulence attributes of infecting species. [4] In Candida spp. dimorphism, adhesions, production of extracellular hydrolases, drug resistance and antigenic modulations are important virulence factors that contribute to pathogenicity. [4]

As most of the studies on virulence factors and host pathogen interactions in Candida spp. are focused on C. albicans, the present study was conducted with an aim to determine the expression of virulence factors of NAC spp. isolated from HIV infected patients with OPC.

Materials and Methods

The present study was conducted in the Department of Microbiology, Rural Medical College and Hospital of Pravara Institute of Medical Sciences, Loni, Maharashtra and is a part of PhD thesis. The protocol of the study was approved by the institutional ethics committee.

A total of 123 NAC spp. isolated from HIV infected patients with OPC were included in the study. The species identification was done by sugar assimilation and colony color on Hichrom Candida agar. HiCandida identification kit supplemented the speciation of isolates. The isolates were preserved at -80 °C in Sabouraud dextrose broth containing 5% glycerol. For each experiment, isolates were subcultured on Sabouraud dextrose agar (SDA) for 48 h at 37 °C.

The virulence factors studied were adherence to buccal epithelial cell (ABEC), exoenzymatic activity (Phospholipase and proteinase) and haemolytic activities.

1) ABEC: Adherence assay was performed as described by Kimura and Pearsall with minor modifications. [5] BECs were collected by gently rubbing the cheek mucosa of healthy laboratory technicians (no signs or symptoms of OPC or other oral lesions and not receiving any antibiotics at the time of study) after obtaining prior consent. As fresh BECs were used, they were collected in the morning on the day of assay. BECs were washed thrice by phosphate buffered saline (PBS) and harvested by centrifugation.

   Equal volumes (1ml) of BEC ($1x10^5$ cells/ml) and yeast suspension ($1x10^7$ cells/ml) were mixed and incubated at 37^0C for 2 h in a shaking water bath at 40 rpm. The mixture was filtered through a 20 µm filter to remove non adherent yeast cells. The BECs on the filter were washed with 5 ml of PBS and finally suspended in 5 ml of PBS. A drop of this suspension was placed on glass slide. The smear was fixed by methanol; air dried and stained with 2% crystal violet for 1 minute. Adherence was determined microscopically by counting the mean number of yeast cells adhering to every 100 BECs. C. albicans ATCC 90028 was used as positive control.

2) Phospholipase production: The isolates were screened for phospholipase production by the method described by Samaranayake et al. [6] by egg yolk agar plate method. Approximately 5 µl of standard inoculum of test strain containing 10^8 cells/ml was aseptically inoculated onto egg yolk agar. After inoculation, the plates were dried at room temperature and then incubated at 35^0C for 3days. The plates were examined for the presence of a zone of precipitate around the colony. The presence of precipitation zone around the colony indicated phospholipase production. Phospholipase activity (Pz) was expressed as the ratio of the colony diameter to the diameter of the colony plus the precipitation zone. A Pz value of 1 denoted no phospholipase activity whereas Pz<1 indicated phospholipase expression by the isolate. C. albicans ATCC 10231 was used as the positive control.

3) Proteinase production: The proteinase activity of the isolates was screened by the method of Aoki et al. [7] with a few modifications, using bovine serum albumin agar (BSA) plates. Approximately 10 µl of standard inoculum containing 10^6 cells/ml was aseptically inoculated onto 1% BSA agar plate. Inoculated plates were incubated at 37^0C for 7 days. Further proteinase activity was inhibited by adding 20% trichloroacetic and the plate was stained with 1.25% amidoblack. The diameter of the colonies was measured prior to staining and the diameter of the clear zones was measured after staining.

   Proteinase index (Prz) was measured in terms of the ratio of the diameter of the colony to the diameter of unstained zone. A Prz value of 1 indicated no proteinase

activity; Prz<1 denoted proteinase expression by Candida isolate. The lower the Prz value, the higher the activity. C. albicans ATCC 10231 was used as positive control.

4) Haemolytic activity: Haemolytic activity of Candida isolates was screened by the method described by Luo et al. [8] Approximately 10 µl of standard inoculum (10^8Candida cells/ml) was inoculated on sheep blood SDA plate. Plates were incubated at 37^0 C in 5% CO_2 for 48 h. The presence of a distinct translucent halo around the inoculum site, viewed with transmitted light, indicated haemolytic activity.

   Haemolytic activity (Hz) was determined by calculating the ratio of the diameter of the colony to that of the translucent zone of haemolysis. C. albicans ATCC 90028 and C. parapsilosis ATCC 22019 were used as positive and negative controls, respectively. One strain each of Streptococcus pyogenes (Lancefield group A) and Streptococcus sanguis, were used as positive controls for beta and alpha haemolysis, respectively.

Result

During the study period, a total of 182 Candida spp. were isolated from oropharyngeal swabs collected from 202 HIV infected individual with oropharyngeal lesions suggestive of candidiasis. Out of these 123 (67.5%) isolates were NAC spp. C. glabrata and C. tropicalis were predominant isolates from NAC spp. C. dubliniensis were isolated from 6 cases (Figure 1).

The virulence factors produced by NAC spp. is shown in Table 1. The capa city of buccal epithelial cell adherence was more in C. dubliniensis (66.6%) isolates. As compared to other NAC spp. C. glabrata demonstrated low ABEC. High phospholipase activity was noted in C. tropicalis (64.1%) followed by C. kefyr (50%). Phospholipase activity was seen in only 1 isolate of C. dubliniensis.

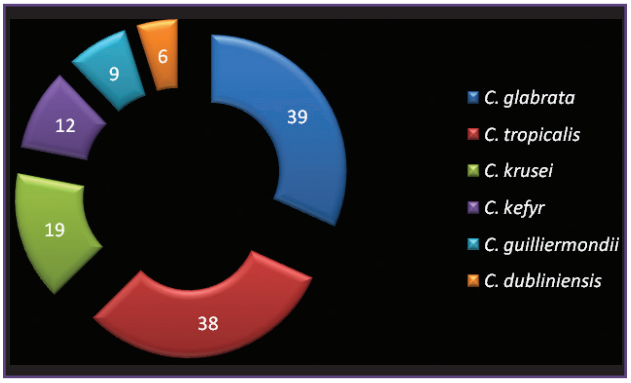

Fig. 1: Species wise distribution of non albicans Candida spp. isolated from HIV infected patients with OPC.

Proteinase activity was high in C. dubliniensis (83.3%) followed by C. tropicalis (60.5%). C. glabrata demonstrated low proteolytic activity as compared to other NAC spp. Haemolysin production was high in C. tropicalis (73.6%) followed by C. kefyr (66.6%) isolates.

Discussion

The emergence of HIV/AIDS has changed the scenario of infectious diseases. Organisms previously considered non pathogenic or less infectious have emerged as potent pathogens. Only few decades back, NAC spp. were overlooked as contaminant or commensals without any pathogenic role and therefore their isolation from clinical specimens was considered as insignificant.

In our study, NAC spp. were the predominant pathogens isolated from HIV infected patients with OPC. C. glabrata and C. tropicalis were the most prevalent isolates. Our finding is consistent with that of Mane et al. [9] Clinical manifestations of NAC spp. infections are similar to that of C. albicans but infections due to NAC spp. are often more treatment resistant. [10] C. glabrata is innately resistant to antifungal drugs and mechanisms of the body. [11] Mucocutaneous candidiasis due to C. glabrata is often

Table 1: Virulence factors production in non albicans Candida spp. isolated from HIV infected patients with OPC.

Candida spp.	Adherence to buccal epithelial cell (%)	Phospholipase activity (%)	Proteinase activity (%)	Haemolytic activity (%)
C. glabrata	14(35.8)	12(30.7)	10(25.6)	21(53.8)
C. tropicalis	19(50)	24(64.1)	23(60.5)	28(73.6)
C. krusei	08(42.1)	09(47.3)	09(47.3)	12(63.1)
C. kefyr	06(50%)	06(50%)	04(33.3)	08(66.6)
C. guilliermondii	04(44.4)	03(33.3%)	03(33.3)	05(55.5)
C. dubliniensis	04(66.6)	01(16.6%)	05(83.3)	03(50)

resistant to azole therapy. Empirical and injudicious use of antimycotics has led to replacement of a commensal, azole-sensitive strain of C. albicans with inherently azole-resistant NAC spp. like C. glabrata and C. krusei, or an azole-resistant strain of C. albicans. [12]

Attributes involved in the adhesions of Candida spp. to host cells is one of the important virulence factor implicated for establishment of infection. Adhesion to host cell is important for colonization and establishment of infection. In our study, C. dubliniensis demonstrated high capability of adherence to human buccal epithelial cell. High adherence capacity in C. dubliniensis was also reported by Gillfillan et al. [13] As compared to other NAC spp. ABEC was lower in C. glabrata isolates. C. glabrata is the only Candida spp. that is haploid and does not form true or pseudohyphae. [11] The low adherence of C. glabrata to buccal epithelial cells might be due to the lack of hyphal transformation. [14]

Extracellular hydrolytic enzymes aid Candida spp. in adherence and invasion of host tissues. [4] Phospholipases facilitate the invasion of host mucosal epithelia by hydrolysing one/more ester linkages in glycerophospholipids. [15] In our study, phospholipase activity was high in C. tropicalis and C. kefyr isolates. High phospholipase activity in C. tropicalis was also noted by Thangman et al. [16] whereas; Samaranayake et al. [6] reported no phospholipase activity in C. tropicalis. These inconsistencies in observations may be due to biological differences among the isolates tested. A review of the available literature has revealed a dearth of information regarding the phospholipase activity in C. dubliniensis. In the present study, phospholipase activity was very less in C. dubliniensis isolates. Low phospholipase activity could be one of the possible reasons for minimal or no capability of C. dubliniensis to cause disseminated infections.

Proteinase plays an important role in pathogenesis of Candida spp. Proteinases facilitate invasion and colonization of host tissue by disrupting host mucosal membranes. It also degrades vital immunological and structural proteins. [17] The ability to express proteinase enzymes not only varies among different species of Candida but also differs among the strains of same species isolated from different body sites. In this study, C. dubliniensis showed maximum proteolytic activity. As compared to other NAC spp. C. glabrata showed low proteinase activity. This may suggest that C. glabrata pathogenesis is mediated by unknown hydrolytic enzymes or virulence factors. [14] This underscores the need of more detailed research to explore the pathogenesis, host-pathogen interaction and other pathogonomic features of this emerging pathogenic Candida spp.

Haemolysin production is one of the important attribute contributing to pathogenicity of Candida spp. It is essential for survival of yeast and is related to the acquisition of iron. [4] In this investigation, haemolytic activity was high in C. tropicalis isolates. Mane et al. [9] reported high haemolysin production in C. tropicalis isolated from HIV infected individuals.

Conclusion

NAC spp. once overlooked as mere contaminants or non pathogenic commensals have emerged as potent pathogens. These isolates are capable of producing virulence factors once attributed only to C. albicans. Knowledge of these virulence factors is important for understanding the pathogenesis of candidiasis and will help to explore new antimycotic drug targets for improved therapeutic regimens.

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Competing Interests

None declared

Reference

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