Non-albicans Candida species: Emergence of neglected pathogens among population of Karachi

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Abstract: Candida albicans was considered as the principal cause of opportunistic candidiasis but nowadays, neglected non-albicans Candida (NAC) species are evolving as more virulent and drug resistant strains. This research was intended to assess pervasiveness of candidiasis mainly caused by NAC species in Karachi city. A total of 562 clinical isolates of Candida spp. collected during the period of one year were identified by microscopic as well as morphological (germ tube formation, characteristics on CHROM agar and Corn meal agar) and Biochemical (sugar assimilation and fermentation) characteristics. Doubtful species were further identified by using Remel RapID™ yeast plus kit. The results were statistically analyzed by SPSS 16.0 version software. Isolated strains of candida revealed slight predominance of C. albicans (54.5%) over non- albicans Candida species (45.5%). Among NAC species, C. tropicalis and C. glabrata were isolated as the predominant species. These clinical species were procured mainly from urine samples of females (73.7%) of age group 20-30 years. No significant correlations exist between Candida species and their months of isolation as well as their isolation from different districts of Karachi. Emergence of NAC species may predict an upcoming threat in health care facilities and hence, require prompt management and accurate identification to suggest empirical antifungal therapy.

Keywords: Candida albicans, non-albicans Candida, opportunistic infections

INTRODUCTION

The greatest risk to human life is not only due to known pathogens but it is more because of unknown and underestimated pathogens. Candida species are the yeast fungi which are a part of normal flora of skin and mucous membrane. Being opportunistic organisms, they are one of the examples of those underestimated enemies which are a serious threat for human health nowadays. Candidiasis, infections caused by these versatile fungi, has been recognized as one of the most common fungal infection, whose clinical manifestations may range from relatively harmless superficial infections to serious systemic life-threatening infections having mortality rate as high as 40% (Hofs et al., 2016). This infection accounts for most of the invasive fungal infections worldwide while in USA it is considered as the fourth leading cause of nosocomial bloodstream infections (Healey et al., 2016). This situation is more alarming in countries of South Asia where the prevalence of candidiasis is almost 20-30 times greater than that in the western countries (Singh and Chakrabarti, 2017). Due to increasing use of broad-spectrum antibiotics and implanted medical devices, nearly all types of manifestations have been increasing and regarding as healthcare-related infections particularly among immune compromised individuals (Uppuluri et al., 2017).

The genus Candida includes nearly 200 species while only 20 species are considered as clinically important (Hofs et al., 2016). Among them, Candida albicans is the most repeatedly isolated specie responsible for causing different manifestations of candidiasis (Chauhan et al., 2019). But during last few years, there has been an alteration in the distribution of these infections and now more cases of candidiasis due to non-albicans Candida have been reported. This changing pattern is more pronounced in South Asian countries where 70-90% cases of candidiasis have been caused by NAC in recent years (Chakrabrti et al., 2015). This epidemiological trend of prevalence of Candida species may also vary from country to country (Saunte et al., 2017). The spectrum of non- albicans Candida species also varies in different geographical locations. As observed in western countries, only C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis and Candida krusei were reported as causative agent of candidiasis. However, in south Asian countries there are more newly emerging species of Candida which accounts for candidiasis. As observed in a study conducted in India regarding candidemia, thirty-one species of Candida were procured from the clinical samples including above mentioned five species (Singh and Chakrabarti, 2017).

The main problem raised with the emergence of these NAC species was associated with their response to
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antimycotic agents used to treat systemic infections. Most of these NAC species revealed reduced susceptibilities to these drugs. This high resistance towards antifungals was not only responsible for the aggravation of the course of disease, but also disseminated these drug resistant isolates among the community (Khadka et al., 2017). In the developed countries, there are many surveillance programs which keep a close check on emergence of such new infectious species but in developing countries like Pakistan such programs are lacking. Even in most of diagnostic laboratories, only C. albicans is identified among all Candida species while other species even isolated from severe systemic candidiasis cases have been reported as non-albicans Candida without any further identification (Ramos et al., 2018).

This research study was aimed not only to assess the emergence of Candida infections in Karachi City but it mainly focused on exploration of non-albicans Candida species responsible for these manifestations. These NAC species are usually not identified up to specie level in most of the diagnostic laboratories of country which not only complicates the process of disease but also fails treatment measures as most of these NAC are resilient to many antimycotic agents.

MATERIALS AND METHODS

Collection of clinical isolates of candida

Five hundred and sixty two isolates of Candida spp. were obtained by convenient sampling from Diagnostic laboratory of Dow University of Health Science (DUHS) during one year (October 2016-September 2017). The details regarding source of isolation, and demography were also recorded from patient’s medical record.

Identification of candida isolates up to specie level

The isolates of Candida were conventionally identified by using following standard diagnostic criteria (Larone, 1995; Mahmoudabadi et al., 2015).

Microscopic examination

The clinical isolates were examined under the microscope by using Gram staining techniques or wet mount technique.

Primary culture

For identification, the clinical isolates of Candida were streaked on Sabouraud’s Dextrose agar (Oxide, UK) for 24 hours at 37°C. The growth characteristics of appeared colonies were recorded.

Germ tube test

This test is used widely to differentiate between C. albicans and C. dubliniensis from Non-albicans Candida species. The isolated colony from fresh culture of Candida was inoculated in sterile human serum (0.5ml) and incubated for 2 hours at 37°C. One drop of serum after incubation was transferred on glass slide and observed for the formation of germ tube under high power (40X) microscope. At least 05 germ tubes in the entire wet preparation were considered as positive result. C. albicans ATCC 14053 was used as positive control while C. glabrata ATCC 9258 was used as negative control.

Characteristics on Chromogenic agar

For the differentiation up to specie level, the Candida isolates were further streaked on Brilliance Candida Agar (Oxoid, CM1007) and incubated at 37°C for 48 hours. The different Candida species produce different colored colonies on this medium such as C. albicans and C. dublinsiens produce light green colored colonies while C. tropicalis produces dark metallic blue colonies. In the same way, C. krusei produces dry irregular pink colored colonies however, other species such as C. kefyr, C. parapsilosis, C. glabrata, C. lusitaniae exhibit yellow, beige and brown colored colonies.

Characteristics on corn meal agar with tween 80

The formation of chlamydospores and blastospores by Candida spp. and their specific arrangement was observed by streaking fresh culture of Candida on corn meal agar (Oxoid CM0103) with tween 80 by using Dalmau’s plate method and incubated at 25°C for 3 to 5 days. The plates were observed directly under 40X lens of microscope.

Physiological/Biochemical characterization

The Candida species were further analyzed for their physiological and biochemical characteristics.

Carbohydrates fermentation test

The ability of carbohydrate fermentation was detected by inoculating colonies of Candida in yeast nitrogen base broth. After incubation, the heavy inoculum (0.1ml) was inoculated in carbohydrates fermentation broth containing inverted Durham tube and 2% sterilized sugar for seven days. The production of acid and gas was observed and recorded (Goel et al., 2018).

Carbohydrate assimilation test

The ability to assimilate carbohydrate was investigated by preparing yeast inoculum (2ml) in yeast nitrogen base (YNB) which was then mixed well with 18ml of melted agar and poured in sterilized petri plates. After solidification of media, filter paper discs of 6mm diameter soaked in 10% sugar solution were aseptically placed on media and incubated for at least three days at 37°C. Presence of yeast growth around the disc indicated assimilation of that particular carbohydrate (Goel et al., 2018).

Identification by using rapid identification kit system

Remia RapID™ yeast plus system kits, a qualitative micro conventional method, was used for the final identification of Candida species which had some doubts.
in identification by above conventional methods. The freshly grown culture of Candida spp., mixed with sterile saline was added in the panel of kit system as per manufacturer’s protocol and incubated for 4 hours at 30°C. Later, reagents A and B (provide with kit) were added according to protocols and the appeared results were correlated with Remel RapID™ yeast plus system computation data base to identify Candida species (Noni et al., 2019).

RESULTS

Collection and Identification of Candida isolates
To assess the emergence pattern of Candida species in cases of candidiasis in Karachi City, five hundred and sixty two isolates of Candida species of clinical origin were procured and identified up to specie level by standard conventional methods. After complete identification, it was revealed that among 562 isolates, 54.5% (306) isolates were of Candida albicans while 45.5% (256) were non-albicans Candida species (NAC). These NAC comprised C. glabrata (16.7%), C. tropicalis (16.5%), C. rugosa (3.8%) and C. krusei (3.9%), C. parapsilosis (1.4%), C. guilliermondii (1.4%), C. kefyr (0.9%), C. zeylanoides (0.5%), C. apicola (0.2%) and C. lipolytica (0.2%) (table 1), fig. 1.

Gender wise distribution of Candida species
The highest numbers of Candida isolates were obtained from females i.e. 414/562 (73.7%) as compared to males 148/562 (26.3%). Among females, C. albicans was isolated as the predominant specie (54.3%) while NAC spp were isolated with slighter rate (45.7%). Among the NAC species, C. glabrata was isolated as predominant spp. In male patients, almost similar distribution of Candida species was observed however, C. tropicalis was isolated as the predominant NAC specie (table 1).

Age wise distribution of Candida species
The Candida isolates were obtained from nine age groups of patients. Among these groups, the overall incidence of Candida spp. was higher in age group 20-30 years which was followed by age group 60-70 years. Interestingly, lesser Candida isolates were obtained from extreme age groups i.e. 0-10 and >80 (fig. 3).

Month wise distribution of Candida species
The Candida isolates were collected throughout the year i.e. from October 2016 to September 2017. The results obtained describe no significant variation in the number of isolations during different months. The statistical analysis also demonstrated a weak correlation (0.523<1) between different months of isolation of C. albicans and NAC species (table 2).
Location wise distribution of Candida species obtained during the study

Candida isolates in this study were obtained from main Laboratory of DUHS having seven collection centers located in six different districts of Karachi City. It has been observed that highest number of Candida isolates were obtained from Karachi Central (32.74%) followed by Karachi East (26.16%) and Karachi South (10.85%) (table 3.1). Moreover, the results revealed almost parallel isolation of C.albicans and NAC species from all districts of Karachi which was also depicted by insignificant relationship between these species and their area of isolation when analyzed statistically (p=0.43>0.05) (table 3).
Clinical specimens and gender wise distribution of Candida isolates

Candida isolates were acquired from different clinical specimens obtained from both genders. In females the highest number of Candida isolates were obtained from urine (53.14%) followed by high vaginal swab (35.2%). However, in males, the Candida species were mainly isolated from urine (65.54%) followed by sputum (13.5%). Interestingly a significant relationship (P = 0.000<0.05) was found between gender and Candida isolates from different clinical specimens (fig. 4).

Fig. 2: Distribution of Candida spp. with respect to gender of patients

Fig. 3: Distribution of Candida species isolated from different age groups of patients

Clinical species wise distribution of Candida species

Different Candida species in this study were obtained from different clinical specimens but the highest no. of these species was obtained from urine (56.4%). The overall isolation pattern exhibited preponderance of C. albicans over NAC spp. from sputum (93%), sterile fluids (78%), pus (56%) and urine samples (54%). While, the NAC species were predominantly isolated from clinical specimens like ear swab (100%), oral swab (60%), stool 100%, medical devices 64%, HVS (54%) as compared to C. albicans. The specie wise isolation from clinical specimens revealed that C. albicans (53.9%), C. tropicalis (19.2%) and C. glabrata (16.1%) were isolated predominantly from urine samples while from high vaginal swab C.albicans (46.2%) was followed by C. glabrata (table 4).
**DISCUSSION**

The unrevealed or underrated pathogens are sometimes more injurious than known pathogens and it was found true with etiology of candidiasis. *C. albicans* has been recognized as the major causative agent of all forms of candidiasis since beginning and the main focus of diagnostic laboratories are always to report this organism as pathogen only while other non-*albicans* Candida species are always considered as unimportant therefore remains unidentified up to specie level. But with time, the epidemiological pattern of candidiasis has been changing and in the recent years the species other than *C. albicans* are also evolving as more virulent and drug resistant species. This emergence of NAC species has been reported from all over the world (Tasneem et al., 2017; Quindos et al., 2018).

This changing trend of candidiasis was also perceived in this study, where *C. albicans* (54.4%) was isolated as the predominant specie as compared to NAC species (45.6%) but this difference was not so marked and therefore isolation of these NAC species can be considered as more or less parallel to *C. albicans*. These findings are in accordance with other studies conducted in Pakistan where *C. albicans* was still the major causative agent of candidiasis but NAC species were also following these rates (Tasneem et al., 2017; Jamil et al., 2017). The study conducted in Turkey and Nepal also supported our findings and reported almost parallel isolation of both *Candida* species (Sharma et al., 2016; Calgin and Cetinkol, 2018). The marked predominance of *C. albicans* over NAC species were reported only from some European countries while from most of other parts of world this pattern has been changed (Lamoth et al., 2018).

In United States, the prevalence of NAC species has been exceeded more than 50% and *C. glabrata* has emerged as predominant pathogen followed by *C. parapsilosis* (Matsumoto et al., 2014; Cleveland et al., 2015; Lamoth et al., 2018). However, in Latin America and Africa this picture was little different and *C.albicans* was still the major pathogenic specie followed by *C. parapsilosis* (Doi et al., 2016). Similarly, in Europe and Australia, *C.albicans* was still appeared as major causative agent but increased prevalence of *C.glabrata* has been reported from all over there (Chapman et al., 2017; Trouve et al., 2017). The shift towards non-*albicans* Candida species was more evident from Asia. The data from India showed that these NAC species (63.2%) were isolated as the predominant pathogen as compared to *C.albicans* (36.8%) and *C.tropicalis* was isolated as the major NAC specie (Deorukhkar et al., 2014). Similar picture was also observed in a previous study where *C.tropicalis* superseded over *C.albicans* when isolated from tuberculosis patients (Naz and Perween, 2004; Bilal et al., 2018) China also presented the similar picture where NAC species superseded over *C.albicans* (Gong et al., 2016). In Japan, the predomination of NAC species was also evident while *C.parapsilosis* was appeared as the most prevalent pathogen (Hirano, 2018). However, from Pakistan, the NAC species such as *C.tropicalis* (38%), *C.parapsilosis* (18%) and *C. glabrata* 16% superseded over *C.albicans* which was isolated from only 12% of adult population (Farooqui, 2013). These findings from Pakistan did not correlate with our findings which might be due to difference in site of isolation of these species.

In the present study, *C. tropicalis* and *C. glabrata* were isolated as predominant species after *C. albicans*. The isolation of *C. tropicalis* was in accordance with many studies and it was reported as major NAC specie from most of Asian countries but isolation of *C.glabrata* in high proportion was unusual because this specie was mostly reported as prominent pathogen from America and Europe (Lamoth et al., 2018). This geographic discrepancy in prevalence of NAC species might be due to certain factors such as chronic illness, use of indwelling medical devices and demographic details of patients (Mohamed et al., 2018). The demographic information obtained during the study also revealed that the species of *Candida* were largely sequestered from female (73.7%) patients as compared to males (26.3%). This high prevalence might be due to their isolation from urine specimens as well as high vaginal swabs. In females, *Candida* species are found as normal commensal of urogenital areas and in certain circumstances these commensals become opportunistic pathogen and cause infections. Therefore, genitourinary candidiasis is one of the most frequent type of candidiasis occurs in females where it usually causes vulvovaginal candidiasis. Even in males these *Candida* species may cause severe genital infections such as balanoposthitis and balanitis. Moreover, candiduria is another prevalent infection caused by these species in both genders (Uppuluri et al., 2017). In the present study, a slightly
higher prevalence of *C. albicans* over other NAC species might be because of the fact that most of the isolates were obtained from urine and high vaginal swab and it was evident that among other species, *C. albicans* has more ability of strong adherence with vaginal and oral mucosa lining (Patel et al., 2012; Vieira de Meloa et al., 2019). This finding is in accordance with previous studies where urine specimens were the main source of isolation of *Candida* species (Khadka et al., 2017, Wiebusch et al., 2017).

**CONCLUSION**

The recent profile of Candida species in this study suggests an upcoming threat by newly arising non-*albicans* Candida species particularly *C. tropicalis* and *C. glabrata* which are already reported as drug resistant species. Therefore, there is a need to manage these non-*albicans* Candida species in population.

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