ORIGINAL RESEARCH PAPER

Microbiology

PREVALENCE OF CANDIDA SPECIES IN VARIOUS CLINICAL SPECIMENS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN IN TERTIARY CARE HOSPITAL: A PROSPECTIVE STUDY.

KEY WORDS: Fungal infections, Candida albicans, Non-albicans Candida, Nosocomial.

Dr. Manjula J Babariya Assistant Professor, Department of Microbiology, Pacific Institute of Medical Sciences (PIMS), Umarda, Udaipur, Rajasthan, India.

Dr. Kamlesh K. Shekhawat* Assistant Professor, Department of Anesthesiology, Critical Care and Pain, Pacific Institute of Medical Sciences (PIMS), Umarda, Udaipur, Rajasthan, India. *Corresponding Author

Dr. Vikram Singh Rathore Assistant Professor, Department of Otorhinolaryngology and Head-Neck Surgery, Pacific Institute of Medical Sciences (PIMS), Umarda, Udaipur, Rajasthan, India.

ABSTRACT

Background: Nosocomial fungal infections have emerged as major pathogen in human being and are associated with high morbidity and mortality despite antifungal therapy. Risk factors for these infections continue to rise in frequency so it is estimated that frequency of these infection will be high in coming decades. Candida species are currently considered as the fourth most common cause of hospital-acquired systemic infections and Candida albicans is generally considered the major pathogen among the species but various study result was in favour of non Candida albicans species. Aim of this study is to identify different candida species in various clinical samples and their antibiotic susceptibility pattern.

Methods and Material: This prospective study was conducted for period of one year from (Nov 2016 to Nov 2017). Total 5400 clinical samples (urine, sputum, ET secretions, blood, etc) were received in microbiology laboratory from various OPD ,Ward and ICU with all aseptic precaution and processed for identification of Candida species and their susceptible antibiotics by use of Sabourad's Dextrose Agar with chloramphenicol antibiotics, CHROM agar, CMA (corn meal agar), SDB (sabourad dextrose broth), YNB (yeast nitrogen base media), Sugar disc (raffinose, sucrose, maltose, lactose), MHA with methylene blue, Antifungal disc – Amphotericin B (100IU), Fluconazole(25µg), Ketoconazole (15µg), Nystatin(50µg), Clotrimazole(10µg), Voriconazole (1µg).

Result: A total of 30 Candida isolates out of 5400 clinical specimens were isolated during study period. Candida was mainly isolated from respiratory samples (12) and urin(7). The most common species of Candida isolated was C. albicans forming 76.66% of the total isolates. The non-albicans candida species form the remaining 23.33% of the total isolates, thus stressing their emergence as major fungal pathogens. Amphotericin B and Nystatin are most effective antibiotic for all isolated species.

Conclusion: The species level identification of Candida is important due to variation in sensitivities of various species to different antifungals and also due to limited therapeutic options because of emergence of resistance to antifungals.

INTRODUCTION

Invasive candidiasis (IC) is a leading cause of mycosis-associated mortality in the United States. The growing number of immunocompromised individuals as a result of the HIV pandemic, use of long-term immunosuppressive therapy in cancer and organ transplant patients, invasive medical procedures, un-sanitary hospital practices and long duration of hospital stay have all favoured the increased incidence of these fungal infections [1,2]. The genus candida includes several species implicated in human pathology. Candida albicans remains the predominant cause of IC, accounting for over half of all cases, but other Candida species also emerged as common cause of IC. Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility. Many of which can exhibit resistance to triazoles and/or amphotericin B. Crude and attributable rates of mortality due to IC remain unacceptably high and unchanged for the past 2 decades. All candida spp. cause diseases ranging from superficial infections such as oral thrush to invasive disease, yet they show differences in disease severity and susceptibility to different antifungal agents [1]. Nonpharmacologic preventive strategies should be emphasized, including hand hygiene; appropriate use, placement, and care of central venous catheters; and prudent use of antimicrobial therapy. Given that delays in appropriate antifungal therapy are associated with increased mortality, improved use of early empirical, preemptive, and prophylactic therapies should also help reduce IC-associated mortality. Candida spp. identification is therefore important for successful management. Speciation helps to understand the epidemiology of candida spp. particularly the source and mode of transmission. This in turn facilitates the development of effective measures to prevent and control transmission of resistant pathogens [1]. This study was undertaken to know the prevalence and to speciate Candida obtained from various clinical samples and their antibiotic susceptibility pattern. Further research to improve diagnostic, preventive, and therapeutic strategies is necessary to reduce the considerable morbidity and mortality associated with IC

Methods and Materials

Study design, study area and sampling process

This prospective cross-sectional study was conducted in tertiary care hospital for a period one year (November 2016 to November 2017) in Udaipur, Rajasthan after ethical committee approval. During study period total 5400 clinical samples including urine, sputum, blood, bronchial wash, body fluid, pus, endotracheal tube secretion (ET sec) were collected from various outpatient department(OPD), WARD and ICU, and send to microbiology laboratory with all aseptic precaution and underwent for further processing.

Data collection and Data analysis

A preformed perform was filled for data collection. Information regarding patients age, sex, clinical sample, clinical diagnosis, SDA broth pattern (surface pellicle, non surface pellicle), Sugar disc results (glucose, sucrose, lactose, raffinose), CHROM agar result, Corn meal agar(CMA) result, Germ tube result, antibiotic sensitivity and resistant pattern. Obtained data were analysed by simple descriptive statistics (i.e. proportions, ratios and percentages). Age of the patients were categorized into 0-20, 21-40, 41-60, > 61years, to determine prevalence of candida species in different age groups.

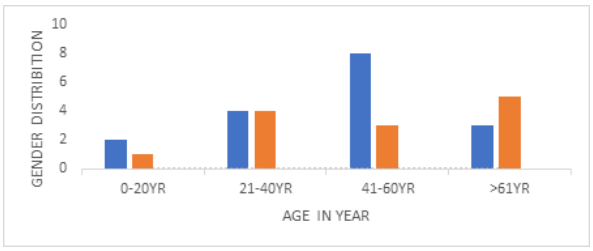
Laboratory procedures

We collected 30 candida isolates among 5400 of total clinical sample during study period. All clinical specimens were processed as per the standard microbiological procedures. They were further speciated by the germ tube test, CHROM agar, chlamydospore formation on CMA and carbohydrate utilization patterns by Sugar Assimilation Tests and for antibiotic sensitivity, we used MHA with methylene blue & commonly available antifungal disc-Amphotericin B (100IU), Fluconazole(25µg), Ketoconazole(15µg), Nystatin (50µg), Clotrimazole(10µg), Voriconazole(1µg).

RESULTS

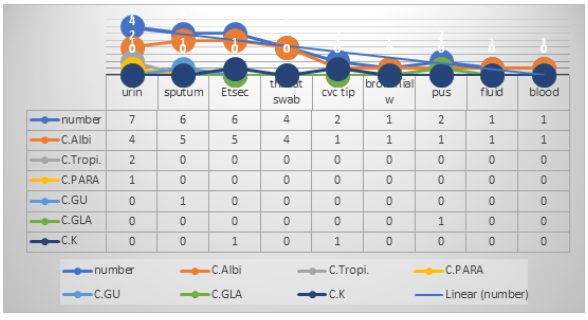
Total 5,400 clinical samples collected during study period of one year, out of them only 30 (0.55%) sample was positive for candida growth. Out of 30 clinical sample 17 (47%) were in male and 13 (43%) in female. Male to female ratio was 1.3:1. Most commonly

affected age group between 41-60 (36%) followed by >61 year (26.66%), 21-40 year (26.66%) [Figure- 1] showing demographic data distribution for positive candida growth.



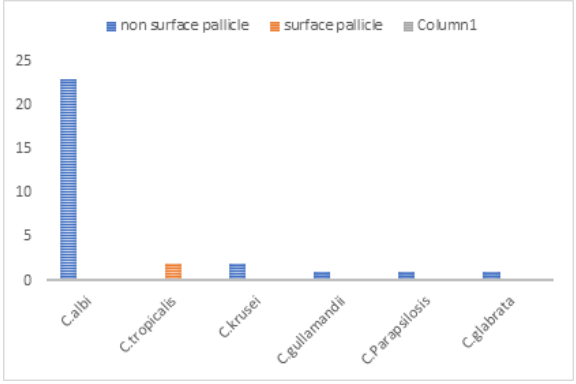
[Figure 1] Demographic data distribution of positive clinical samples

Various clinical samples including urine, sputum, endotracheal tube secretion (ET sec.), throat swabs, bronchial wash, central venous catheter tip (CVC tip), blood, wound pus, body fluid were collected. Maximum positive candida growth found in urine 7 (23.33%), followed by sputum 6 (20%) and ET secretion 6 (20%), throat swab 4 (13.33%), CVC tip 2 (6.66%), pus 2 (6.66%), blood 1 (3.33%), bronchial wash 1 (3.33%), body fluid1 (3.33%). Most common isolated species was Candida albicans in 23 (76.66%), followed by Candida tropicalis 2 (6.66%), Candida krusei 2 (6.66%), Candida parapsilosis 1 (3.33%), Candida glabrate 1 (3.33%), Candida guilliermondii 1 (3.33%) [Figure2]



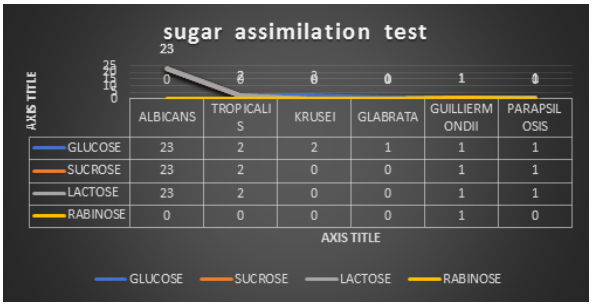
[Figure 2] showing species distribution of candida species in various clinical samples. [C.Albi-Candida albicans, C. Tropi-Candida tropicalis, C.PARA- Candida Parapsilosis, C.GU-Candida guilliermondii, C.GLA-Candida glabrate, C.K-Candida krusei].

We found non surface pellicle growth in 28(93.33%) candida species, and 2(6.66%) was surface pellicle on sabouraud dextrose agar(SDA). Surface pellicle species was C.tropicalis and out of 28 non surface pellicle ,23(82.14%) was C.albicans,2(7.14%) was C.krusei,1(3.57%) was C.guilliermandii,1(3.57%) was C.parapsilosis,1(3.57%) was C. glabrate[Figure 3].



[Figure 3] SDA Broth distribution of various candida species.

Candida albicans species was showing positive result for glucose, sucrose and lactose in all 23(100%) isolated cases and negative for raffinose sugar in all cases. Candida tropicalis(100%) was also positive for glucose, sucrose, lactose and negative for raffinose. Candida krusei showing positive for glucose and negative for sucrose, lactose and raffinose in another 100% of cases. Candida guilliermondii positive to glucose, sucrose, lactose, raffinose in 100% of cases and C. parapsilosis positive for glucose, sucrose, lactose and negative for raffinose in 100% of cases and candida glabrate was positive only for glucose in 100% of cases and negative for other 3 sugar [Figure4].



Sugar disc growth distribution of various candida species [Figure4]

CHROM agar and CMA and germ tube was used for species identification. According to our result C. albicans was showing light green colonies, C.glabrata and C.krusei showing pink colonies, C.tropicalis showing blue colonies, C. parapsilosis and C. guilliermondii showing violet colonies on CHROM agar. Germ tube test positive for all C. albicans (100%) and negative for other species (C. krusei,C.tropicalis,C.parapsilosis, C.galabrata, C.guilliermondii). Candida species are very common nosocomial fungal infection in recent era and mortality rate is also high so we try to established method of species identification and their antibiotic sensitivity pattern. We used commonly available antibiotic like Amphotericin B, Nystatin, Ketoconazole, Clotrimazole, Fluconazole, Voriconazole. Most of candida species are sensitive to amphotericin B, followed by Nystatin. Candida albicans is most common strain isolated 23(76.66%) and most sensitive to Amphotericin B,18(78.20%), followed by Nystatin, 14(60.86%), clotrimazole,13(56.52%), and fluconazole and voriconazole are equally effective,11(47.82%). Next most common species are candida tropicalis (6.66%) and candida krusei (6.66%), and C.tropicalis showed 100% sensitivity to amphotericin, clotrimazole,fluconazole,voriconazole,50% sensitive to Nystatin and ketoconazole. Other pathogenic species showed 100% sensitivity to amphotericin and nystatin only. [Table 1]

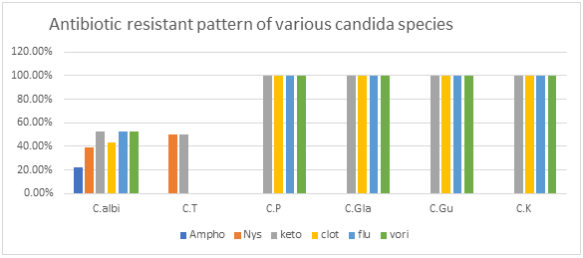
Antibiotic sensitivity pattern of various candida species [Table 1]

species	Ampho	Nys	Keto	Clot	Flu	Vori
C.al(23)	18 (78.2%)	14 (60.86%)	11 (47.82%)	13 (56.52%)	11 (47.82%)	11 (47.82%)
C.T(2)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)
C.P(1)	1 (100%)	1 (100%)	0%	0%	0%	0%
C.Gu(1)	1 (100%)	1 (100%)	0%	0%	0%	0%
C.Gl(1)	1 (100%)	1 (100%)	0%	0%	0%	0%
C.K(2)	2 (100%)	1 (50%)	0%	0%	0%	0%

[C.al-candida albicans, C.T-candida tropicalis, C.P-Parapsilosis, C.Gu-candida guilliermondii, C.Gl-candida glabrate, C.K-candida krusei].[Ampho-amphotericine,Nys-Nystetine,Keto-ketoconazole, Clot-clotrimazole,Flu-flucanazole,Vori-voriconazole]

C. albicans resistant to voriconazole, fluconazole, ketoconazole in 52% of cases and 21.7% for amphotericin B, 39.13% for Nystatin, 43.47% for clotrimazole and C.tropicalis resistant to ketoconazole(50%), and Nystatin(50%),other isolated species

showed high resistant to fluconazole, ketoconazole, voriconazole, and clotrimazole.[Figure 5]



[Figure 5] Ampho- Amphotericine, Nys-Nystatine, Keto-ketoconazole, Clot-clotrimazole,Flu-flucanazole, Vori-voriconazole]

DISCUSSION

Numerous factors have contributed to the increase incidence of fungal infections. The most important is an ever-expanding population with immunocompromise due to mucosal or cutaneous barrier disruption, defects in the number and function of neutrophils or in cell-mediated immunity, metabolic dysfunction, and extremes of age [3]. Increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies, and transplantation further increases the risk for both common and uncommon opportunistic fungi [4,5,6]. In addition, as our aging population becomes increasingly mobile, environmental exposures to a variety of endemic fungal pathogens become more common and may further increase the risk of mycotic disease. The list of opportunistic fungi causing serious, life-threatening infection increases every year. In addition to *Candida*, *Aspergillus* and *Cryptococcus* species, the opportunistic fungi include non dematiaceous or hyaline moulds, and the pigmented or dematiaceous fungi [4,5,6,7]. Despite this formidable list of opportunistic fungi, without question the single most important cause of opportunistic mycoses worldwide remains *Candida*. In present study, a total 5400 various clinical specimens were collected during study period of one year. Among them 30 (0.55%) candida isolates were found. These isolates was mainly isolated from respiratory samples (sputum, ET sec) 12(40%) followed by urine 7(23.33%), throat swab 4(13.33%), central venous tip catheter 2(6.66%), pus 2(6.66%), bronchial wash 1(3.33%), blood 1(3.33%), body fluids 1(3.33%). And in our study candida albicans was the commonest species isolated causing 23(76.66%) of the infections followed by 7(23.33%) was non C. albicans species in which C. tropicalis & C.krusei causing (6.66%) and (6.66%) of the infections respectively. Our study is in agreement with the studies conducted by Mohandas et al.in (2011) who found C. albicans to be commonest isolates [9]. Dharwad S et al., also found same result and Amphotericin B was most effective antifungal. and Mokad das et. al., (39.5%) which all found same results [10,11]. Data from surveillance and control of pathogens of epidemiological importance (SCOPE) surveillance system confirms that Candida species have become the fourth leading cause of blood stream infections. A recent study (2007) by MN Chowta et. al [8] shows that Candidemia is associated with increased cost and attributable mortality of 38%. Although Candida albicans is the most frequently encountered organism, a number of reports have documented non-albicans Candida species such as C. tropicalis, C. glabrata, C. parapsilosis and C. krusei and other filamentous fungi as emerging pathogens in recent years. There are few Indian studies regarding the incidence and risk factors for candidemia. This study also throws light on the prevalence of candidemia and invasive candidiasis in a tertiary care hospital which is not only associated with a significant mortality but also extends the duration of hospital stay and cost of medical care [8] A study by Chakrabati A showed non-albicans Candida to have a higher incidence (75%) than C. albicans (25%). These findings seem to suggest that non-albicans Candida are emerging as important pathogens [12]. The speciation of Candida is important to identify the incidence and trends of Candida infections in a given set-up of study. It is also essential for the choice of antifungals because of variation in the sensitivity of different species to different antifungals. In our study C.albicans is most common strain 23(76.66%) and most sensitive to Amphotericin B,18(78.20%), followed by Nystatin,14(60.86%), clotrimazole,13(56.52%),and fluconazole and voriconazole are equally effective,11(47.82%). Next most common species are candida tropicalis (6.66%) and candida krusei (6.66%), and C. tropicalis showed 100% sensitivity to amphotericin, clotrimazole, fluconazole, voriconazole,50%sensitive to Nystatin and ketoconazole. Other pathogenic species showed 100% sensitivity to amphotericin and nystatin only. This finding is similar with study done by Maria F.et al.,1998 and study conducted by Ananth Kashid et al.,2011 [13,14]. The azoles being effective against C. albicans and C. tropicalis, are found to be ineffective against C. krusei and C. glabrata [15,16]. Though candidiasis can occur at all ages, studies by Dalal PJ and Kelkar SS at Mumbai showed the highest incidence of candidiasis to be in the age group of 21-40 years [17]. Our study also had similar findings, most commonly affected age group between 41-60 (36%)followed by >61 year (26.66%) with male preponderance. A study conducted by Patel et.at.,[18], and other study done by Jaggi et al.[19] recorded a male preponderance. However candiduria was higher in females than in males. However, in a study by Kandhari KC et. al., the incidence was found to be higher in females (61.2%) than in males (38.8%) [20].

CONCLUSION

We conclude that in recent years increasing in predisposing factors for opportunistic infections which is associated with high morbidity and mortality, the species level identification of the pathogenic isolates along with their antibiotic susceptibility patterns can greatly influence the treatment options for the clinician and may have an impact on the patient care. Candidemia and invasive candidiasis in common infection in recent era with 40%mortality along with resistant pathogenic species to antibiotic. Candida albicans is most predominant species and other Candida species also common, and all they are highly sensitive to Amphotericine B, and in some cases with Nystatin. In our study invasive candidiasis common in male. Further research require to evaluate predisposing factors for male preponderance and others effective antifungal drug.

Conflict of interest: The authors have no conflict of interest to declare.

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