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STUDY OF *CANDIDA SPECIES* ISOLATED FROM DIFFERENT CLINICAL SPECIMENS

Microbiology

Smita Mohod M.B.B.S.(Post Graduate student M.D. Microbiology)

Ravindra K Khadse* M.B.B.S.,M.D.(Microbiology)Associate Professor Dept of Microbiology IGGMC,Nagpur,Maharashtra,India*Corresponding Author

Sonal P Chavan M.B.B.S.,M.D.(Microbiology) Assistant Professor Dept of Microbiology IGGMC,Nagpur,Maharashtra,India

Sharmila S Raut M.B.B.S.,M.D.(Microbiology) Professor and Head of Dept of Microbiology IGGMC,Nagpur,Maharashtra,India

ABSTRACT

Background : Infections by Candida species have increased medical importance over the past few decades. It is the major cause of morbidity due to overuse of broad spectrum antimicrobials which enhance the opportunity to cause the disease hence accurate and early identification and study of drug resistance is necessary as an effective measure for the management of patients. **Objectives:** To isolate, identify and to determine antifungal susceptibility pattern of Candida species. **Methods:** A Total of sixty-six Candida species isolated from various specimen were studied from Jan 2019 to April 2019.Identification and Antifungal susceptibility of Candida species was performed with conventional and Vitek 2 Compact using ID and AST YS08 kits respectively. **Results:** In this study, out of 66 clinical specimen, Candida species was most commonly isolated from sputum 23(34%) followed by urine 13(20%). Candida non-albicans 36 (55%) was found to be more common than Candida albicans 30(45%). Out of 36 Candida non-albicans species, C. tropicalis was most common species 13 (36%) followed by C.krusei 8(22%). Maximum antifungal sensitivity was found for Voriconazole, Caspofungin, Micafungin while least sensitivity was found for Fluconazole. **Conclusion:** This study concludes that species level identification of Candida species and their antifungal sensitivity testing should be performed to achieve better clinical results And To Be Updated With Changing Trend Of Antifungal Sensitivity.

KEYWORDS

Antifungal susceptibility, Candida Isolation, Candida species.

INTRODUCTION

Candida is a commonest human fungal pathogens that cause both mucosal and deep tissue infections [1] *Candida* species is a normal flora of human beings but in presence of predisposing factors they can cause different types of infections . It can cause infection either by lowering the host defence mechanism or altering the balance of normal microbial flora. Infections by *Candida* species have increased medical importance over the past few decades. It is the major cause of morbidity due to overuse of broad spectrum antimicrobials which enhance the opportunity to cause the disease hence accurate and early identification and study of drug resistance is necessary as an effective measure for the management of patients [2] So, this study was performed with an objective to isolate and to identify various *Candida* species from different clinical specimens and to determine antifungal susceptibility pattern of various *Candida* species.

MATERIAL & METHODS

Study design : Prospective observational study.

Study place : Department of Microbiology, Indira Gandhi Government Medical college and Hospital, Nagpur.

Duration of study : Four months Jan 2019 to April 2019

Sample size : A Total of 66 *Candida* Isolates Were Isolated from various specimen (sputum, blood, urine, vaginal swab, Bronchoalveolar lavage, endotracheal secreations, pus) were studied from OPD, IPD and ICU of hospital proceeded for laboratory investigation.

Inclusion criteria : Patients with various age group are included in this study predominantly focus given on immuno-compromised, endocrine disorders also history of chemothearapy and smoking.

Exclusion criteria: Isolates of samples not showing pure growth are excluded. The isolates diagnosed to be fungus other than *Candida species* were exempted from the study.

Study was approved by institutional Ethics committee . For the clinical significance of *Candida* isolates from sputum and urine, the specimens were analysed by microscopy as well as for the evidence of budding yeast cell with pseudohyphae along with significant pus cells . The preliminary diagnosis of specimens were performed by wet mount, KOH mount, Gram stain, culture on Sabouraud dextrose agar (SDA). Identification was made by standard conventional tests. Gram stain was performed from direct samples and inoculated on Sabouraud dextrose agar supplemented with Chloramphenicol incubated at 37°C for 24 hours. Any visible growth seen on SDA slope was processed for identification of the species. The yeasty, pasty and creamy colony on SDA slope showed Gram positive budding yeast cells with on microscopic examination. Germ tube test was done and positives were identified as *C .albicans* or *C. dubliniensis* . *C.albicans* was further identified by growth at 45°C and chlamydospore formation on cornmeal agar **(Dalmau plate Technique)** Simultaneously, isolates were inoculated on CHROM agar[4] (Hi-media,India) incubated at 37°C for 24 hrs *Candida* species were differentiated based on type of the growth and colour of isolates on CHROM agar. Identification and Antifungal susceptibility of *Candida* species was further confirmed with Vitek [2] Compact using ID and AST YS08 kits [6] respectively.

Figure 1 : *Candida species* were differentiated based on type of the growth and colour of isolates on CHROMagar

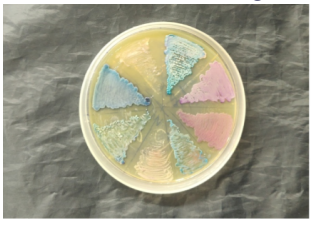
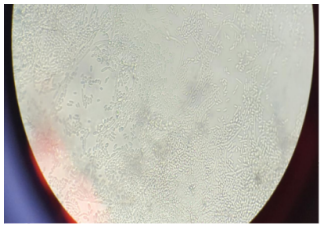


Figure 2 : Dalmau Plate Technique Is Printed Twice Please Remove This Heading From Here As It Is Already There Above This Picture



RESULTS

A total of 66 C*andida* Isolates were isolated from various clinical samples. Isolates of *Candida* were predominantly found in male patients (56%) than female patients(44%). Out of this 66 *Candida* isolates, 27 (56.06 %) cases were Immunocompromised while 16(24.24%) cases were having endocrine disorders , 9(13.63%) cases had received chemothearapy, 4(6.06%)cases had history of smoking. Isolates from IPD samples were 38(57.57%), OPD samples were 23(34.84%) and ICU 5(07.57%)Isolates were obtained from various samples such as sputum 23 (34.8%), Urine 13(19.7%) ,Blood 13 (19.7%) Vaginal swab 09(13.6%), Endotracheal tube secretions 03(04.5%),Bronchoalveolar lavage 03(04.5%),Pus02(03.0%)(Table 1). Species identification revealed that *C.albicans* was the major species to be isolated which constitutes 20 (45%). Other species isolated were *C.tropicalis* 13(19.7%),*C.krusei* 09(12.1%), *C.paraspilosis* 07(10.6%), *C.glabrata*04(6.06%), *C.gullermondi* 02(4.54%), *C.keyfer* 01(1.51%) (Table 2).On antifungal susceptibility testing our study revealed (Table 3) that all isolated *Candida* species were 100 % sensitive to Caspofungin, and Micafungin. The antifungal susceptibility for Amphotericin B all isolated *Candida* species were 100 % sensitive except *C. albicans* which was 93 % sensitive. Similary for Flucytosine *C.albicans* was 87 % sensitive where as all other isolated *Candida* species were 100 % sensitive . Antifungal suceptibility testing of Voriconazole showed that *C.albicans ,C.tropicalis, C.paraspilosis, C.gullermondi* and *C. keyfer* were 100 % sensitive but *C.krusei* and *C. glabrata* were totally resistant to voriconazole. AST of Fluconazole showed that *C.albicans* was 89 % sensitive . *C.tropicalis , C. paraspilosis C.gullermondi* and *C. keyfer* were 100 % sensitive where as *C.krusei and C. glabrata* where totally resistant to Fluconazole .

Table 1 : Sample wise distribution of *Candida* species

Type of sample	Number (%)
Sputum	23 (34.8 %)
Urine	13 (19.7 %)
Blood	13 (19.7 %)
Vaginal swab	09(13.6 %)
Endotracheal tube secretions	03 (04.5 %)
Bronchoalveolar lavage	03 (04.5 %)
Pus	02 (03.0 %)
TOTAL	66 (100 %)

Table 2 : Species wise distribution of Various isolated *Candida* species

Candida species	Percentage
C. albicans	30 (45%)
C. tropicalis	13 (19.7%)
C.krusei	08 (12.1%)
C.paraspilosis	07 (10.6%)
C.glabrata	04 (6.06 %)
C.keyfer	01 (1.51 %)
Total	66 (100 %)

Table 3 : Antifungal sensitivity of isolated candida species to various Antifungal drugs

Antifungal agents	*C. albicans*	*C. tropicalis*	*C.krusei*	*C.paraspilosis*	*C.glabrata*	*C. gullermondi*	*C.keyfer*	Total
Voriconazole	100 %	100 %	0 %	100 %	0 %	100 %	100 %	71%
Fluconazole	89 %	100 %	0 %	100 %	0 %	100 %	100 %	55.5 %
Caspofungin	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
Micafungin	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
Amphotericin B	93 %	100 %	100 %	100 %	100 %	100 %	100 %	99 %
Flucytosine	87 %	100 %	100 %	100 %	100 %	100 %	100 %	98.14 %

DISCUSSION

Incidence of *Candidiasis* has been increasing vigorously which have the direct impact on choice of imperic antifungal thearapy and clinical outcome. In our study, males (56%) predominated females (44%) which is comparable to Kumar et al [7].

In our study , the most frequently isolated species was *C. albicans* 45 %.Comparative study of different *Candida* species isolated in their studies by different workers showed that *C. albicans* isolation was highest in each of them except Chakrabarti et al , who reported *C. tropicalis* as the highest (42 %) followed by *C. albicans (*25%). Some other studies Prasad et al ,Vijaya et al also showed comparable results with that of ours [9].

Knowledge of species distribution and drug sensitivity pattern among species help the clinician to choose early empirical therapy. In the present study, most common *non-albicans Candida* species was *C. tropicalis* 13(19.7%) which was higher than rates reported by Baradkar et al [8] (17.94%), but lower than that showed by Vijaya et al (35.29 %)[9]. Other non-albicans *Candida* species representing *C. krusei* 08(12.1%) *C.paraspilosis* 07 (10.6 %) *C.Glabrata* 04 (6.06%) *C.Guillermondi* 03(4.54%) and *C. keyfer 01* (1.51%) found in this study were also comparable with other workers.

In our study 66 *Candida* isolates were obtained over 4 months duration, majority of *Candida* species 30 (45 %) were isolated from sputum 23(34.8 %) followed by blood(19.7 %) and urine 13 (19.7 %). On the other hand Khadka et al [3]. isolated a total of 100 *Candida* speceis over 7 months of period from different clinical specimens. Majority of *Candida* species were isolated from urine (48%), sputum (42%), catheter tip (4%), blood (2%), high vaginal swabs (2%) and endotracheal tube (2%)

In our study antifungal susceptibility testing revealed that *Candida* species showed maximally sensitivity to Caspofungin, Micafungin, and most resistant to fluconazole and sensitivity to Flucytosine and Amphotericin B is 87 % and 93% respectively. And antifungal sensitivity of *C. tropicalis , C.krusei* to Fluconazole is 100 % and 0 % respectively . As per Khadka et[3] al. Antifungal susceptibility profile of *Candida* species to Fluconazole was found to be 71.5% susceptible (S).

CONCLUSION

Due to injudicious use of antifungal medications,the resistance among *Candida* species is increasing ,which leads to increasing risk of life threatening *Candida* infections. This study concludes that species level identification of various *Candida* species and their antifungal sensitivity testing should be performed to achieve better clinical results And To Be Updated With Changing Trend Of Antifungal Sensitivity.

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Conflicts of interest

No conflict of interest amongst authors.

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