



## “ANOTHER CHALLENGE- RISING INCIDENCE OF CANDIDA INFECTION IN PATIENTS WITH COVID 19”

### Medical Microbiology

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### ABSTRACT

Symptoms of COVID 19 and some of the fungal diseases are similar like fever, cough, respiratory distress, abdominal pain, malaise etc. So, we need strong support of all hospital infection control team and laboratory testing methods to differentiate between these two. People affected with SARS CoV2 virus, admitted in critical care units are more prone to develop fungal infections and among the several fungal agents, Candida species are raising their heads leading to increase in morbidity and mortality. So, awareness is necessary for prevention as well as diagnosis and treatment of the fungal infections among the COVID 19 infected patients. The objective of this study is to estimate the prevalence of various Candida species as well as to increase the awareness for prevention of secondary fungal infections in COVID positive patients.

#### MATERIALS AND METHODS

Samples were collected from COVID positive patients from January 2021 to December 2021. After the initial identification by Gram stain and wet mount preparation, culture was done on MacConkey agar, Modified HiCrome UTI agar, SDA agar and Blood agar followed by routine incubation. VITEK2 Compact System was the method of choice for identification and antifungal sensitivity.

#### RESULTS

Among the total 144 collected samples, 35 samples showed positive growth of Candida species. Among the various collected samples, highest (25 %) positivity seen in urine samples and Candida albicans was the most prevalent (35%) species. All Candida species showed decreased sensitivity towards Fluconazole.

### KEYWORDS

COVID 19, Fungal infection, Candida species, VITEK2 Compact, antifungal sensitivity.

#### INTRODUCTION

COVID 19 (Corona virus disease 2019) caused by SARS – COV 2 (severe acute respiratory syndrome corona virus 2) is a novel enveloped RNA Beta coronavirus that was first identified in Wuhan City by Chinese Centre for diseases control & prevention in (Dec2019). SARS - Cov2 mainly transmitted by inhalation of Droplets (Direct spread), & indirectly by touching the contaminated surfaces & fomites & then again touching the mucosa of our nose, eyes etc.

The virus exerts its pathogenesis by targeting the ACE2 receptors present in the surface of Type II pneumocytes of lung epithelium, binds with its spike protein (S). During the course of host – virus interaction type-2 transmembrane protease in virus cleaves the S1/S2 domain of the viral spike protein & promotes the viral entry within the target cells. After entering, virus exerts immunomodulation in the form of dysfunction of CD8+ T-cells which plays a important role by their cytotoxicity in viral infected cells.

Moreover there are cytokine storm in moderate to severe cases with higher levels of Pro – inflammatory cytokines like IL-2, IL-6, TNF- $\alpha$  & decreased levels of anti-inflammatory cytokines IL10, decreased CD8, increased CD4/CD8 Ratio, lymphopenia all predispose to invasive fungal infections.

Virus mediated manipulation in lung parenchyma is considered to be the major mechanism resulting in viral pneumonias following covid 19. Like other viral pneumonias, bacterial & fungal co – infections are common complications in Covid 19 patients. Frequently seriously ill Covid 19 patients with ARDS admitted to ICU with invasive monitoring allows entry of opportunistic fungus. Due to prolonged ICU stay or hospital stay patients may develop HAI (Hospital Associated infections) with opportunistic fungus<sup>[1]</sup>.

In addition, mild to moderate cases receive immunosuppressant medication such as systemic corticosteroids, Tocilizumab, Cyclosporine that are in one hand fundamental to stop ‘‘Cytokine Storm’’ that occurs in severe cases. Application of these drugs potentially increases the risk of severe pulmonary and other infections with opportunistic mycosis.

Invasive fungal infections ranged between 4% to 14% in Covid positive patients has been by opportunistic fungus like Mucor mycoses, Aspergillosis (CAPA) has been reported widely<sup>[2]</sup>.

Fungal UTI are increasing infrequency with the clinical features of asymptomatic urinary tract colonization to cystitis or severe pyelonephritis.

Candida is a yeast like fungus occurs as commensals in oral, Gastrointestinal, skin, Genitourinary tract mucosal surfaces. Most commonly occurred endogenous pathogen causing superficial mucosal infections like oral thrush but can cause systemic mycosis in immunosuppressed condition. In Covid patients predisposing factors for colonized as well as invasive Candida infections are various comorbidities like Diabetes, Hypertension, COPD with repeated use of inhalers, prolonged use of antibiotics, systemic steroids, use of immunomodulator drugs, prolonged ICU stay with invasive monitors, under mechanical intubations and indwelling urinary catheters<sup>[3]</sup>.

Due to prolonged ICU stay many of the critically ill Covid 19 patients colonized with Candida. Out of these 5% - 40% develop invasive Candidiasis in the form of Candidemia contributed to significant mortality.

Worldwide Candida *albicans* is the most commonly isolated species, but Non albicans Candida has now become a challenge for the clinicians. Amongst Non Albicans Candida, Candida *glabrata* is less susceptible & Candida *krusei* is intrinsically resistant to Fluconazole. Candida *tropicalis* are also resistant to many antifungals as well as potential for bio film formation. So not only to determine Candida Co infection but also speciation is of immense importance for appropriate antifungal therapy in Covid 19 Associated Systemic Candidiasis (CAC).

The objective of this study is to estimate the prevalence as well as speciation of candida isolates & to bring out various risk factors associated with Candidiasis in Covid positive patients.

#### MATERIALS AND METHODS

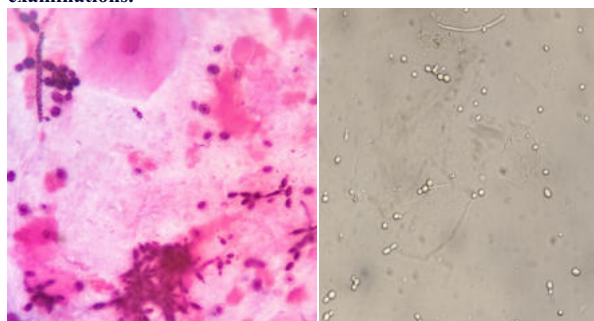
This observational study was conducted between January 2021 to December 2021. 144 Samples were collected from RTPCR confirmed SAR CoV2 Positive symptomatic patients to rule out secondary infections. Microscopic examinations were carried out first by Direct Wet mount preparations, KOH preparation and Gram stain. After initial identification, samples were inoculated on MacConkey's agar, HiCrome UTI agar, Sabourads dextrose agar (SDA) and Blood agar and samples with Positive growth of Candida species were selected

and further identification and antibiotic sensitivity were done by VITEK 2 Compact Machine taking Yeast ID and YS08 AST Cards.

## RESULTS

Total 144 samples from COVID positive patients were collected among them 35 samples showed growth of *Candida* species. Urine samples first screened by Direct Wet Mount preparations which showed budding yeast cells and pseudohyphae and initial screening of sputum samples were by Gram's stain. We followed Bartlett's grading for sputum quality checking and samples with quality score of +1, +2 and +3 were reported and we found variable numbers of budding yeast cells and pseudohyphae. Inoculated samples after 24-48 hrs. of incubation showed white to cream colored, dry to pasty colonies of *Candida* species. Among the all-clinical samples we received, urine samples showed highest number of *Candida* species (25%) followed by sputum samples (24%) and Endotracheal aspirate (22.7%).

**Figure 1. Initial identification of the samples by microscopic examinations.**



**Gram Stain Of Sputum**

**Wet Mount Preparation Of Urine**

**Table:1 Distribution of *Candida* species in various clinical samples**

CLINICAL SPECIMEN	TOTAL NO OF SAMPLES	NO OF CANDIDA SPECIES ISOLATED	PERCENTAGE OF CANDIDA SPECIES
URINE	64	16	25%
SPUTUM	58	14	24%
ENDOTRACHEAL ASPIRATE	22	5	22.7%

Culture positive *Candida* species were confirmed by Automated VITEK 2 Compact System. It showed maximum no of isolated species is *Candida albicans* (37%). Next common isolated species was *Candida tropicalis* (31%), followed by *Candida krusei* (20%) and *Candida parapsilosis* (11%)

**Table:2 Distribution of *Candida* species in clinical specimens**

DIFFERENT SPECIES	TOTAL ISOLATES	PERCENTAGE
<i>Candida albicans</i>	13	37%
<i>Candida tropicalis</i>	11	31%
<i>Candida krusei</i>	7	20%
<i>Candida parapsilosis</i>	4	11%

Sensitivity of the isolated species showed an alarming scenario. The commonly used antifungal Fluconazole is almost resistant to all isolated species. Other five drugs, Capsfungin, Amphotericin B, Voriconazole, Micafungin and Flucytocin showed variable sensitivity pattern and *Candida tropicalis* showed resistance to many antifungals.

**Table:3 Percentage of Antifungal susceptibility results of *Candida* isolates by VITEK 2 Compact System**

Antifungal agents Sensitivity	<i>C. albicans</i>	<i>C.tropicalis</i>	<i>C.krusei</i>	<i>C.parapsilosis</i>
Fluconazole	59%	45%	0	51%
Capsfungin	97%	86%	96%	99%
Amphotericin B	100%	98%	100%	100%
Voriconazole	99%	86%	96%	100%
Micafungin	98%	90%	98%	99%
Flucytocin	99%	95%	97%	98%

## DISCUSSION

From our study it is evident that *Candida* infection of respiratory and genitourinary tract is increasing in hospitalized COVID positive patients. We received various samples, among them urine samples

showed highest positivity (25%) followed by sputum samples (24%) and ET aspirate (22.7%). *Candida albicans* species was the maximum isolated species (37%).

Automated Sensitivity method (VITEK 2COMPACT) gave a clear picture of the antifungal sensitivity of the isolated *Candida* species. It is clear from the study that we are losing a good antifungal agent i.e Fluconazole.

Reasons for the higher disease frequency are unclear. The higher rate of *Candida* infection may reflect an accumulation of risk factors, such as prolonged ICU stays, protracted invasive mechanical ventilation, ECMO, broad-spectrum antimicrobial use, renal replacement therapy, and the presence and duration of central venous catheters and urinary catheters. Breaches in routine infection prevention practices during the pandemic may have also played a role, including crowded hospital rooms, decreased staff-to-patient ratios, the limited availability of personal protective equipment, and changes in cleaning and disinfection practices. Breach in infection prevention practices are likely reasons leading to outbreaks of *Candida auris* in Florida (United States) and throughout the globe<sup>[4]</sup>.

Similar study was conducted on 2020 in UK by S Hughes et al showing high positivity of *Candida* species in COVID 19 positive patients.

Another study conducted by Akshay Raut on June 2021, showing rising incidence of fungal infections in COVID patients.

## CONCLUSIONS

During the pandemic, there has been an increase in the reporting of fungal infections associated with COVID-19. Rates of invasive *Candida* infection in the ICU are above pre- COVID 19 era level. In many cases, risk factors for invasive fungal infections and appropriate diagnostic strategies have been challenging to define, which has led to increased incidence of secondary fungal infections. So, awareness is necessary among clinicians for early detection and treatment of all opportunistic fungal infections in COVID patients and also reducing the risk factors responsible for various hospital acquired opportunist infections. And above all, strong support of hospital infection control team and management system is required to prevent occurrence of opportunistic *Candida* or other fungal infections.

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