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COVID-19 ASSOCIATED FUNGAL INFECTIONS, A DEFINITE BOON FOR MYCOLOGY LABORATORIES - AN EXPERIENCE FROM A TERTIARY CARE HOSPITAL IN PUNJAB, INDIA.

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ABSTRACT: Fungal infections constituted a major chunk of secondary infections in COVID-19 due to immunosuppresion and rampant use of steroids. Invasive Aspergillosis, *Candidemia* and COVID associated mucormycois (CAM) post second wave in India among COVID-19 patients was a challenging experience for the clinicians. Increased burden of fungal infections led to a tremendous increase in the sample load of the mycology laboratories across the country. This retrospective study was planned to observe the types of fungal infections reported during COVID-19 pandemic and study the impact of COVID-19 on the sample burden in the mycology laboratory of our tertiary care hospital. Samples received from admitted patients in mycology laboratory were processed using standard procedures. The data was collected and analysed in terms of change in the sample numbers, sample types received during pre and post COVID years (Year 2019 to Year 2022) and also the types of fungal infections reported during pandemic. Total number of samples received in the year 2019, 2020, 2021 and 2022 were 656, 396, 940 and 1276 respectively while the fungal positivity reported was 6.5%, 11.8%, 23.22% and 22.02% in the consecutive years. Sputum remained the predominant sample type in all four years. Candidemia followed by invasive aspergillosis and CAM were commonly reported fungal infections during COVID-19 pandemic. Mucormycosis along with other COVID-19 associated fungal infections have proven to be a boon for mycology in India. Increased awareness among treating clinicians has increased the sample burden in mycology laboratories.

INTRODUCTION: Emerging viral respiratory infections are becoming a major medical concern and are leading causes of morbidity and mortality across the world. These are often complicated by bacterial or fungal infections which may sometimes lead to fatal outcomes [1]. The co-pathogenesis of respiratory viral and fungal co-infections is characterized by complex interactions and involves a dynamic interplay between the virulence of co-infecting pathogens and the host immune defences [1]. COVID-19 infection had caused serious damage to the upper and lower respiratory tracts and can lead to secondary infections from bacteria, other viruses, and fungi through immune system modulation [2].

Among secondary fungal infections, invasive *Candida* infections were the initial infections described among COVID-19 patients admitted in ICU caused by *Candida albicans* mainly with few outbreaks of *C. auris* reported globally [2]. Early 2021, saw the emergence of COVID19-associated pulmonary aspergillosis (CAPA) reported from China and that was reported globally thereafter. During the second wave of the COVID-19 pandemic, India saw an unprecedented rise in cases

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of COVID-19 associated mucormycosis (CAM) and >47,500 cases were reported between May 2021 to August 2021 [2]. Uncontrolled diabetes, and systemic corticosteroid (over) treatment were the two important risk factors implicated for the development of CAM in India and other countries. India's fungal burden is high but still is underappreciated in clinical practice [1]. The current study was planned to observe the impact of COVID-19 on the total sample burden and observe any variations in the samples types received in the mycology laboratory from year 2019 to year 2022.

MATERIAL AND METHODS: The present retrospective study was conducted by the Department of Microbiology of our tertiary care hospital in Patiala. All the samples received in the mycology laboratory both from outpatients and inpatients of Rajindra hospital, Patiala from January 2019 to December 2022 were included in the study. The samples received were processed in the mycology laboratory using standard mycological techniques [3].

The direct microscopic examination with 10% potassium hydroxide (KOH) wet mounts was performed and the fungal culture was performed on Sabouraud's Dextrose Agar (SDA; Hi-Media, Mumbai, India) in duplicate and incubated at 25°C and 37°C. The fungal identification was made by macroscopic and microscopic examination by lactophenol cotton blue (LCB) wet mount of the fungal growth. The information on the samples received in mycology laboratory from January 2019 to December 2022 was collected and analysed to observe for any change in the number of samples received, the sample types and change in the profile of fungal strains isolated.

RESULTS: The total number of samples received in the mycology laboratory were 656, 396, 940 and 1276 respectively in the years 2019, 2020, 2021 and 2022. The rate of fungal positivity observed was 6.5% (n-43) in year 2019, 11.8% (n-47) in 2020, 23.22% (n-223) in 2021 and 22.02% (n-281) in the year 2022 **Fig. 1.**

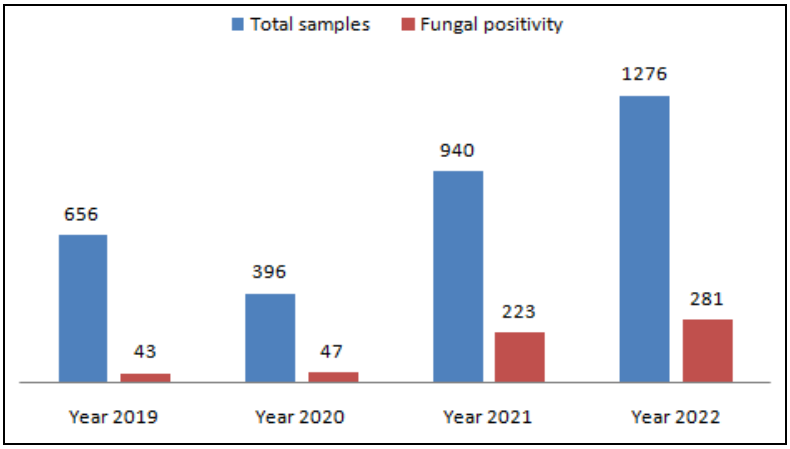

FIG. 1: SHOWS THE SAMPLE BURDEN IN MYCOLOGY LABORATORY AND THE FUNGAL POSITIVITY (BY KOH/CULTURE) OVER THE FOUR YEARS (2019-2022)

The different types of samples received during the four years are given in **Table 1.**

TABLE 1: SHOWS THE VARIOUS SAMPLE TYPES RECEIVED IN MYCOLOGY LABORATORY FROM THE YEARS 2019-2022

Sample type	Year 2019 n (%)	Year 2020 n (%)	Year 2021 n (%)	Year 2022 n (%)
Sputum	254 (38.71%)	156 (39.39)	301 (32.02)	425 (33.31)
Urine	123 (18.75)	108 (27.27)	241 (25.63)	331 (25.94)
BAL	45 (6.85)	27 (6.81)	105 (11.17)	97 (7.60)
Tissue	16 (2.43)	5 (1.26)	13 (1.38)	53 (4.15)
Pus	19 (2.90)	6 (1.51)	11 (1.17)	34 (2.66)
Nasal/oral swabs	26 (3.96)	25 (6.31)	184 (19.57)	156 (12.22)
Hair/ Nail/Skin	87 (13.26)	10 (2.52)	14 (1.49)	48 (3.76)
Corneal scrapings	23 (3.50)	13 (3.28)	7 (0.74)	21 (1.65)
Other Body fluids	63 (9.60)	46 (11.61)	64 (6.80)	111 (8.70)
Total	656	396	940	1276

Sputum and urine samples remained the highest in number in all four years, while samples like BAL, nasal swabs and tissue biopsy samples showed an increase during the years 2021 and 2022. As far as fungal positivity is concerned, a drastic increase in the mucormycosis cases was observed between 2021 and 2022, although fungal infections due to septate fungi too demonstrated an absolute increase in numbers after the year 2020 **Fig. 2.**

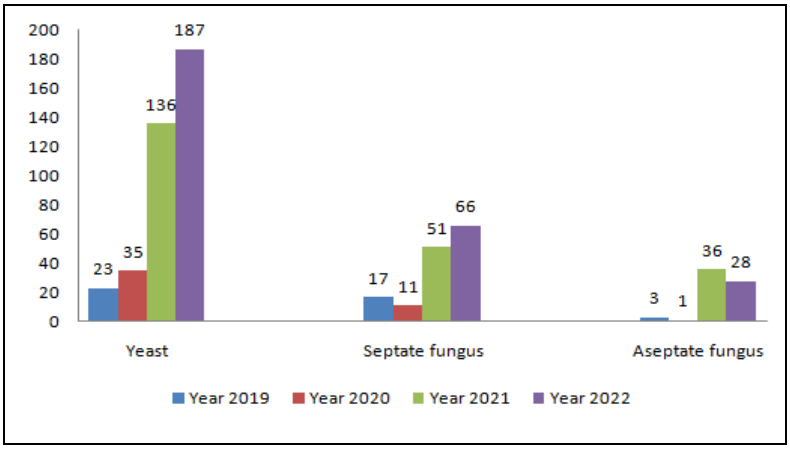

FIG. 2: SHOWS THE FUNGAL PROFILE ON KOH OF FUNGAL POSITIVE SAMPLES IN THE YEARS 2019-2022

DISCUSSION: It is estimated that out of 5 million fungal species that exist today, around 300 are associated with human infections [4]. Invasive fungal infections (IFIs) pose the biggest challenge to the clinicians as far as their management is concerned especially amongst critically ill and immune-compromised patients [5]. During COVID-19 pandemic, the healthcare services across the globe took a hit, with hospitals facing unprecedented challenge to manage an unexpectedly large number of SARS-CoV2 patients requiring intensive care services [6]. The secondary fungal infections such as invasive candidiasis were being reported in COVID-19 patients since the first wave but the second wave of COVID-19 brought along with it a rare and emerging fungal infection *i.e.* mucormycosis. The major risk factors associated with this infection were uncontrolled diabetes that was already prevalent in India and the steroid use that was frequently used for the management of COVID-19 patients [7]. India being a world capital of Diabetes mellitus was a major reason for such a dramatic rise of CAM cases being reported from our country that had shaken the already burdened healthcare system during pandemic [8]. Proportionately, it led to an increased sample load in the microbiology laboratories, already performing the diagnostic testing for COVID-19, secondary bacterial and various associated fungal infections. During the influenza pandemics too, around 25% of bacterial and fungal infections were reported among the patients [9]. We in our hospital too, had experienced a large increase in samples received in the mycology laboratory during COVID-19 pandemic, and it continued thereafter. However, there was an initial dip observed in the numbers of samples received in the year 2020 as compared to previous years, a massive increase in sample number was noted post second wave of COVID-19 which coincided with the CAM epidemic of CAM in India. Over the next years, the number of samples received in the years 2021 and 2022 almost doubled as compared to previous years. Though not reported much during first wave of COVID-19, invasive *Candidiasis* became the forerunner as the COVID pandemic progressed especially in the patients admitted in critical care units, of particular concern were the outbreaks of severe *Candida auris* infections [10]. The risk factors responsible for invasive candidiasis in ICU patients included the use of broad spectrum antibiotics, central venous catheters and corticosteroid use [7]. The incidence of candidemia reported was much higher in studies from Asian Continent (2.5%-23.5%) [11-13] as compared to European countries like Spain, Italy and United Kingdom where the incidences of candidemia were 0.4%, 8%, and 12.6%, respectively [14-16]. Frequent outbreaks of *C. auris* were reported from ICUs in many countries such as United States of America, China, Mexico, and Lebanon in COVID-19 patients [17]. In India, an ICU setting in New Delhi, had reported 2 out 15

candidemia cases to be caused by *C. auris* [10]. Our centre had reported 4, 9, 17 and 20 cases of candidemia respectively in the years 2019, 2020, 2021 and 2022 but due to use of conventional blood culture and identification systems, we could not identify the occurrence of *C. auris* in our ICU settings. Cases of CAPA started emerging within few months of the SARS-CoV2 pandemic [1]. The most common *Aspergillus* species causing coinfection in COVID-19 patients was *A. fumigatus* followed by *A. flavus.* The cardinal risk factor for developing CAPA was the structural lung damage caused in the patients with underlying pathology such as chronic obstructive pulmonary disease (COPD) or asthma besides the use of corticosteroids, broad-spectrum antibiotics in severe COVID patients [7].

There were wide variations in the incidence rates of CAPA reported across the globe probably due to the diagnostic difficulties in differentiating *aspergillosis as* potential complication of COVID-19 from apre-existing infection in COVID-19 patients with underlying chronic pulmonary disease [9]. In a systemic review, the prevalence rates of CAPA was observed to be 10% but it differed in many studies due to variation in surveillance practices and the case definitions used [18]. A high rate of mortality was seen among CAPA patients but the survival was found to be better when antifungal therapy was administered early [19].

We observed a rise in fungal infections caused by septate fungal hyphae in the year 2021, where in *A. flavus* was commonly isolated species from respiratory tract samples (n=40 out of 156). The combined use of clinical assessment and histopathological examination of respiratory specimens collected from critically ill patients have been found to be helpful in diagnosis. Nowadays, diagnostic criteria have further been improved with the incorporation of tests for *Aspergillus* specific antigens galactomannan (GM) and 1,3-β-D glucan (BDG), along with fungus culture [9]. Post second wave of COVID-19, India saw an unprecedented rise in number of COVID associated mucormycosis (CAM) cases. There has been a consistent rise in the prevalence of mucormycosis in India over the years which is almost 70 times more as compared to global data [20]. During COVID-19 pandemic, the pooled prevalence of CAM was reported to be 50 times higher (7/1000 patients) than the background prevalence (0.14 cases/1000 patients [21]. Risk factors for CAM occurrence include poorly controlled diabetes mellitus and the use of systemic corticosteroids. In one large retrospective observational study from India, studying the COVID-19 associated rhino-orbital cerebral mycosis observed that 87% of affected patients had received corticosteroids, and 78% suffered from diabetes with 14% mortality rate [22]. In our tertiary care hospital, as compared to previous years, a sudden increase in number of mucormycosis cases was observed in the years 2021 (n=36) and 2022 (n=28) coinciding the CAM outbreak in India (Fig. 2) especially among the patients with the history of COVID-19 infection where *Rhizopus* species was the most commonly isolated.

In India, no holistic research has been conducted to estimate the true burden of fungal infections in a nation of >1.3 billion people [2]. It was during COVID-19 pandemic that the Government of India realised the importance of keeping the fungal registry looking at the pre-COVID and post-COVID rates of fungal infections [2]. CAM shook the nation by surprise during the second wave of COVID-19 that GOI declared a mucormycosis epidemic on 10 May 2021 and made it a notifiable infection especially when it was noted that CAM showed more than 14% fatality rate during COVID pandemic [2].

In December 2022, for the first time ever the list of priority fungal pathogens threatening public health has been released by WHO [23]. Arecent study has suggested that about 4.1% of the Indian population is probably affected with serious fungal infections which the authors have reported to be >10 times the burden of tuberculosis in India [2]. Our medical college too, observed similar trends in the fungal positivity especially after CVOID-19 pandemic. The number of mucormycosis cases too showed an increase, post second wave of COVID-19 in line with the national rates. The probable reason for the this rise in sample burden as well as fungal positivity is the increased awareness of the clinicians about the emerging fungal infections such as candidemia and mucormycosis which they encountered in large numbers during COVID-19 pandemic. There is a dire need to conduct training programmes for medical, biomedical, pharmacy

and public health specialists to recognize invasive fungal infections (IFIs) at an early stage [2]. With continued attention of the global medical fraternity, we may be able to equip ourselves well in a fight against these emerging fungal infections.

CONCLUSION: Mucormycosis along with other COVID 19 associated fungal infections have proven to be a boon for mycology in India. The sensitisation and increased awareness about rare fungal infections among treating clinicians has, on one hand increased the sample burden in mycology laboratories while on the other hand have improved the patient's management in terms of early diagnosis and hence the administration of timely antifungal therapy.

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