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"Antifungal susceptibility testing of Aspergillus isolated from nasal washings of Post Covid patients in tertiary care hospital"

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ABSTRACT

Background: *The omnipresent fungi Aspergillus species are commonly found in soil, water, and decaying waste. Aspergillosis is a systemic fungal infection that can affect equally immunocompetent as well as immunocompromised individuals. A contagious disease called Coronavirus disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCov-2). In Wuhan, China, in December 2019, authorities discovered the first reported case. Aspergillosis has also occurred in people with severe respiratory infections (such as flu or COVID-19). An intensive care unit (ICU) patient with severe COVID-19 is highly susceptible to bacterial and fungal infections. These fungal co-infections are becoming more common and can lead to serious disease and even death. The present study aims to evaluate antifungal susceptibility testing of Amphotericin B, Itraconazole, and Voriconazole on isolates of Aspergillus isolated from nasal washings of post COVID patients.*

Methods: *A total of 300 samples of nasal washings were collected and screened from post COVID patients. The susceptibilities of the isolates to three different antifungal agents were tested by using Microbroth dilution method.*

Results: *65 isolates were identified as Aspergillus and their susceptibilities to antifungal agents Amphotericin B, Itraconazole and Voriconazole were done. All the isolates showed good sensitivity towards the three antifungal agents.*

Conclusion: *Aspergillus was one of the commonest fungal isolates obtained from nasal washings of post COVID patients. All sixty-five isolates showed good sensitivity to three antifungal agents which are the usual drug of choice used for Aspergillus infection. Hence complications can be prevented by regular follow up and antifungal therapy wherever required.*

Keywords: *Aspergillus, COVID, Antifungal susceptibility.*

INTRODUCTION:

The omnipresent fungi *Aspergillus* species are commonly found in soil, water, and decaying waste. Systemic aspergillosis is carried on by the spores entering the lungs and spreading from there. Aspergillosis is a systemic fungal infection that can affect equally to immunocompetent as well as immunocompromised individuals. Infections with *Aspergillus* in humans were first discovered in the eighteenth century. Micheli made the first attempt to identify the genera Aspergillus in 1729[1], which had been later approved by Hallerin 1768[2] and Fries in 1832[3]. A contagious disease called coronavirus disease 2019 (COVID-19) is brought by severe acute respiratory syndrome coronavirus 2 (SARSCoV-2). In Wuhan, China, in December 2019, authorities discovered the first reported case. Since then, the illness has spread globally, causing an ongoing pandemic [4]. Aspergillosis has also occurred in people with severe respiratory infections (such the flu or COVID-19) [5]. An intensive care unit (ICU) patient with severe COVID-19 is highly susceptible to bacterial and fungal infections. Patients with COVID-19 are more likely to develop invasive candidiasis or aspergillosis as fungal infections. These fungal co-infections are becoming more common and can lead to serious disease and even death [6]. At present we study the antifungal susceptibility of Aspergillus isolated from nasal washings from post COVID patients.

Material and method: Nasal washings were collected from post COVID patients. A total 65 isolates were from nasal washings of post COVID patients of MGM Hospital in Kamothe, Navi Mumbai. The samples were inoculated on SDA and KOH mount and calcofluor staining was done. The fungal isolates were identified by LPCB and slide culture. All Aspergillus isolates were subjected to Antifungal susceptibility testing using Micro dilution method.

ANTIFUNGAL SUSCEPTIBILITY TESTING USING MICRODILUTION METHOD [7].

The method is based on CLSI approved document, M27-A3 micro dilution methodology for moulds.

A broth micro dilution method adopted from the M27-A micro dilution methodology for moulds was used. RPMI 1640, RPMI 1640 supplemented with to 2 % glucose (RPMI-2) and antibiotic medium 3 supplemented to 2 % glucose (AM3) was used as test media. MICs were observed for Aspergillums isolates. The MIC was obtained for the fungicidal [8].

Results:

Table 1: Fungal profile of nasal washings received from Post COVID Patients (n=300)

Isolates	Positive	Percentage
Aspergillus	65	21.66%
Mucor	13	4.33%
Candida	60	20%
Penicillium	1	0.33%
Trichosporon	1	0.33%
Fusarium	3	1%
Streptomyces	2	0.66%
Sterile	155	51.66%
Total	300	100%

Table 2: Showing gender-wise incidence of *Aspergillus*.

Sex	No. of positive (n=65)	Percentages
Male	51	78.46%
Female	14	21.53%
Total	65	100%

Figure 1: Showing different species of *Aspergillus*.

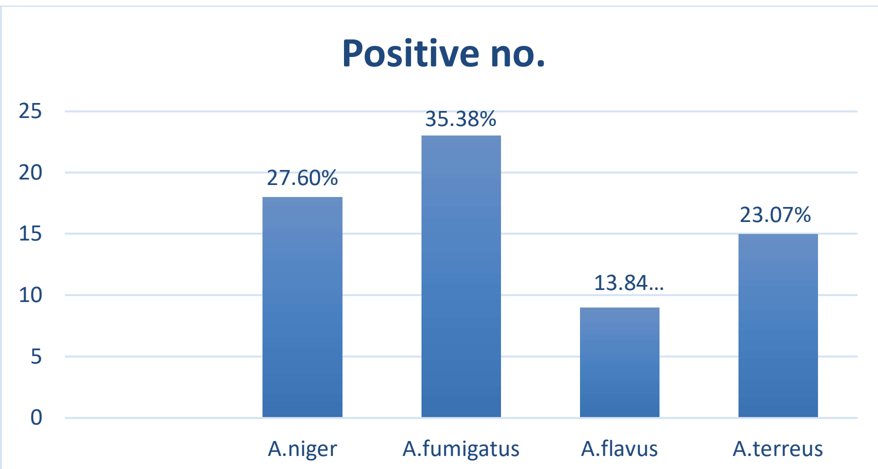

Table 3A: Showing GM MICs and ranges of amphotericin B after 24, 48 and 72h.

Species	No. of isolates	Geometric mean (range) MIC (µg/ml)		
		24h	48h	72h
A.flavus	9	0.86(0.25-2)	1.11(1-4)	1.44(1-4)
A.fumigatus	23	0.78(0.5-1)	1.32(1-4)	1.63(1-4)
A.niger	18	0.25(0.06-0.5)	0.36(0.125-1)	0.52(0.25-1)
A.terreus	15	0.06(0.06-0.125)	0.06(0.06-0.125)	0.03(0.03-0.06)

Table 3B: Showing GM MICs and ranges of itraconazole after 24, 48 and 72h.

Species	No. of isolates	Geometric mean (range) MIC (µg/ml)		
		24h	48h	72h
A.flavus	9	0.26 (0.06-0.5)	0.29 (0.06-0.5)	0.32 (0.125-0.5)
A.fumigatus	23	0.3 (0.06-0.5)	0.4 (0.125-2)	0.45 (0.0125-1)
A.niger	18	0.29 (0.06-1)	0.40 (0.06-1)	0.52 (0.06-1)
A.terreus	15	0.15 (0.03-0.5)	0.25 (0.03-0.5)	0.25 (0.06-0.5)

Table 3C: Showing Geometric mean (GM) MICs and ranges of Voriconazole after 24, 48 and 72h.

Species	No. of isolates	Geometric mean (range) MIC (µg/ml)		
		24h	48h	72h
A.flavus	9	0.19 (0.06-0.5)	0.25(0.125-1)	0.30 (0.125-1)
A.fumigatus	23	0.3 (0.125-0.5)	0.3 (0.125-0.5	0.4 90.125-1)
A.niger	18	0.20 (0.06-0.5)	0.30 (0.06-0.5)	0.40 (0.06-1)
A.terreus	15	0.18 (0.06-1)	0.25(0.06-1)	0.35 (0.25-1)

DISCUSSION:

Rate of incidence of *Aspergillus spp.* in post COVID patients were 21.66%. The findings of the present study correlate with that of **Lansbury L *et.al*.** who reported the prevalence of *Aspergillus* in post covid patients of 19.6% to 33.3% [9].

In this study maximum number of *Aspergillus* were isolated from OMFS (63.07%) ward followed by HDU (13.84%), MICU (12.30%) and ENT OPD (10.76%). In a study by **Erlangga Y. *et.al*,** it was seen that prevalence of *Aspergillus* in the patients admitted in ICU was 10% to 28.8% [10].

In our study Amphotericin B MICs have shown relatively narrow range for *Aspergillus.* MICs of *A.fumigatius*and *A.flavus* were the highest followed by other species. MICs for *A.niger* were the lowest.

Itraconazole MICs were distributed in a narrow range. MICs rose to >16 µg/ml at 48h for all isolates. The lowest itraconazole MICs were obtained for *A.niger.* The antifungal susceptibility pattern of our isolates correlates with the pattern studied by **Arikan. *et. Al*,** where clinical isolates of Aspergillus were tested [11].

The voriconazole MICs for *Aspergillus* isolates covered a narrow range with no prominent difference between the species. In a study by **Kirchhoff. *et.al,*** the antifungal susceptibility testing results (MIC range) for itraconazole and voriconazole was 0.25 – >8 µg/ml, 0.06 - >8 µg/ml, 0.25 - 8 µg/ml respectively [12].

CONCLUSION:

From the present study we can conclude that although Mucormycosis was considered as a major fungal pathogen in patients who had suffered from COVID 19, in our study we saw that Aspergillus was isolated in significant number from patients especially during second wave. We also saw that all Aspergillus isolates showed good sensitivity to Itraconazole, Amphotericin B and Voriconazole which are the usual drugs of choice used for Aspergillus infection.

Hence regular examination of nasal washings of post COVID patients can help in picking up different fungal isolates in time so that appropriate treatment and intervention. Strategies can be planned, which will help in reducing further complications and decrease morbidity and mortality in patients. In case of Aspergillus infection common antifungal drugs like Amphotericin B, Itraconazole and voriconazole can be given safely as good sensitivity of all isolates were seen to these drugs.

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