Fungal assessment and Covid-19; a retrospective study from Hyderabad, Pakistan

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Abstract

This study aims to conduct fungal assessment of the hospital in Hyderabad, Sindh and retrospective study of Covid-19 patients who were admitted in (ICU), who might be at risk of developing nosocomial infections. 15 different locations were examined from October to November 2021. Air samples were collected with active and passive methods. Malt extract agar was used as sampling media for the fungal assessment. Air samples were collected in 2 intervals from 10 am to 2 pm and from 12 pm to 4 pm for the period of 2 months. For retrospective study the data of 8 patients was obtained from hospitals who were admitted in (ICU). Fungal colonies were checked on 4th and 6th day and results of both methods active and passive were compared and increase in fungal colonies was observed on 6th day and more colonies were recorded in evening interval. Retrospective study highlighted that two patients were diagnosed with fungal infection cause mortality and it also highlights the need for comparative studies for the need for screening for fungal infections in severe COVID-19 patients with certain risk factors. This study depicts retrospective study of Covid-19 patients to conduct fungal assessment which will help to take safety in health care systems.

Keywords: *Fungal Assessment; Air Sampling; Nosocomial Infections; Covid-19 CAPA; ICU Admission.*

1. Introduction

Aspergillosis complicating intense influenza infection has been more and more observed and detected globally. According to studies, in covid-19 sufferers a new strain of Covid associated pulmonary aspergillus was also diagnosed and it was different from normal aspergillus because it made complications in patients who did not have healthy immunity [11]. Around the globe in various countries it caused many deaths in patients who were admitted in Intensive care Units ICU and it is a nosocomial infection. The most dominant pathogens that are found in hospital and natural environment are Bacteria and Fungi. The presence of these organisms is mishandled, and environmental monitoring is not carried out on daily basis [1]. It caused infections in people who does not have a healthy immune system. Aspergillus fumigatus belongs to family of fungal species and is most dominating dangerous organism found in indoor as well as outdoor environment [1]. Aspergillus fumigatus mostly results in invasive aspergillus and causes deaths.

Current studies have shown that aspergillosis have caused 50% mortality rate in Covid-19 patients especially in India where black fungus was emerged as health concern for covid-19 patients [14]. The diameter of most fungal spores fall in range of 2–10 um, allowing smooth access in airways of the human breathing tract [3], from where contamination by means of pathogens and their subsequent dissemination is possible .

Prolonged stay of Patients in ICU was more at danger for getting infected by Covid associated aspergillus infections were not monitored at regular basis that made them to be getting infected by this infection.[4] There has been significant studies on screening and diagnosis of aspergilloses in covid-19 patients. This study is being conducted to investigate post covid-19 impacts on patients.

2. Research Materials and Methods

2.1. Research Instruments

Two sampling methods were used to examine the fungal colonies of a hospital.

2.1.1. Active Air Method

Spin air sampler for active method with flow rate of 100 L/min by direct impaction for fungal concentrations was used

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to collect air samples from 15 locations from a hospital in Hyderabad.

2.1.2. Passive method

Petri dishes were settled on ground to collect air samples.

2.2. Media preparation

Malt extract agar was used as sampling media for the fungal assessment. 35.6 gram was suspended in 1000ml of distilled water and media was autoclaved at 121℃ for 15 minutes. After autoclaving, media was placed in water bath at 45℃. Prepared media was poured into sterilized petri dishes(90mm) under laminar hood.

2.3. Air sampling

Air samples were collected with active and passive methods. Fifteen (15) different locations were examined from October to November 2021 in two (2) hospital in Hyderabad, Sindh. Malt extract agar was used as sampling media for the fungal assessment. Each sample was taken with the frequency of 10 minutes with SPIN AIR sampler with the flow rate of 100L/minutes and incubated at 250C. Air Samples were collected in 2 intervals from 10 am to 2 pm and from 12 pm to 4 pm for the period of 2 months. Air samples were incubated at 250C for 7 days.

Fig. 1: Air Sampling

Fig. 2: Media Preparation

3. Results

Fungal colonies were counted on J-2 colony counter. Fungal colonies were checked on 4th and 6th day and results were compared. Fungal colonies were expressed in colonies per Petri dishes. Various conditions are associated with increase in fungal concentrations. Results of both methods were compared. Increase in fungal colonies on 6th day of incubation was recorded throughout the research work. Paediatrics ward recorded more fungal colonies followed by main gate. More fungal colonies were also observed on the afternoon session. Intensive care unit also had the significant number of colonies hence it can be said that patients who are admitted in hospitals are at a great risk for developing nosocomial infections. Active methods recorded more colonies than passive method for the whole duration.

Table 1: Active Method (Time: 10 am to 2 pm-October)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	55	62
2	ICU Surgical	42	45
3	ICU Surgical -from patient's bed	42	54
4	ICU Medical	38	54
5	COVID-19 Isolation Room	42	45
6	Operation Theatre (After operation)	35	48
7	Operation Theatre during Operation	45	68
8	Paediatrics	76	89
9	Waiting Area	72	95
10	ICU – PEADS	68	80
11	Emergency unit	38	45
12	Nephrology Unit	49	53
13	Thalassemia	41	44
14	Gynaecology	39	43
15	Main Entrance	115	144
	Total colonies	797	969

Table 2: Passive Method (Time: 10 am to 2 pm-October)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	36	40
2	ICU Surgical	34	42
3	ICU Surgical -from patient's bed	34	37
4	ICU Medical	24	29
5	COVID-19 Isolation Room	28	33
6	Operation Theatre (After operation)	27	29
7	Operation Theatre during Operation	32	39
8	Paediatrics	54	59
9	Waiting Area	40	44
10	ICU – PEADS	34	39
11	Emergency unit	25	36
12	Nephrology Unit	36	43
13	Thalassemia	24	30
14	Gynaecology	30	35
15	Main Entrance	65	85
	Total colonies	523	620

Table 3: Active Method (Time: 12 pm to 4 pm-October)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	45	53
2	ICU Surgical	55	58
3	ICU Surgical -from patient's bed	38	45
4	ICU Medical	25	30
5	COVID-19 Isolation Room	38	43
6	Operation Theatre (After operation)	49	54
7	Operation Theatre during Operation	88	95
8	Paediatrics	65	69
9	Waiting Area	61	65
10	ICU – PEADS	66	71
11	Emergency unit	52	57
12	Nephrology Unit	67	72
13	Thalassemia	69	77
14	Gynaecology	51	56
15	Main Entrance	95	110
	Total colonies	864	955

Table 4: Passive Method (Time: 12 pm to 4 pm-October)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	26	29
2	ICU Surgical	32	35
3	ICU Surgical -from patient's bed	34	39
4	ICU Medical	24	27
5	COVID-19 Isolation Room	22	29
6	Operation Theatre (After operation)	29	32
7	Operation Theatre during Operation	31	35
8	Paediatrics	68	73
9	Waiting Area	66	70
10	ICU – PEADS	46	55
11	Emergency unit	34	39
12	Nephrology Unit	27	32
13	Thalassemia	25	29
14	Gynaecology	32	35
15	Main Entrance	62	85
	Total colonies	558	644

Table 5: Active Method (Time: 10 am to 2 pm-November)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	85	99
2	ICU Surgical	110	122
3	ICU Surgical -from patient's bed	52	78
4	ICU Medical	74	79
5	COVID-19 Isolation Room	93	114
6	Operation Theatre (After operation)	64	81
7	Operation Theatre during Operation	88	92
8	Paediatrics	110	125
9	Waiting Area	66	89
10	ICU – PEADS	66	87
11	Emergency unit	66	87
12	Nephrology Unit	78	95
13	Thalassemia	72	77
14	Gynaecology	73	86
15	Main Entrance	115	143
	Total colonies	1212	1454

Table 6: Passive Method (Time: 10 am to 2 pm-November)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	65	68
2	ICU Surgical	55	59
3	ICU Surgical -from patient's bed	35	39
4	ICU Medical	62	68
5	COVID-19 Isolation Room	82	87
6	Operation Theatre (After operation)	53	57
7	Operation Theatre during Operation	72	77
8	Paediatrics	88	93
9	Waiting Area	55	58
10	ICU – PEADS	41	46
11	Emergency unit	46	48
12	Nephrology Unit	57	59
13	Thalassemia	45	49
14	Gynaecology	51	58
15	Main Entrance	62	72
	Total colonies	869	938

Table 7: Active Method (Time: 12 pm to 4 pm-November)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	93	105
2	ICU Surgical	113	129
3	ICU Surgical -from patient's bed	55	65
4	ICU Medical	79	85
5	COVID-19 Isolation Room	72	86
6	Operation Theatre (After operation)	68	76
7	Operation Theatre during Operation	65	83
8	Paediatrics	105	133
9	Waiting Area	73	78
10	ICU – PEADS	85	95
11	Emergency unit	83	98
12	Nephrology Unit	91	104
13	Thalassemia	83	93
14	Gynaecology	95	101
15	Main Entrance	135	155
	Total colonies	1295	1486

Table 8: Passive Method (Time: 12 pm to 4 pm-November)

S. No:	Location	Colonies on 4th day	Colonies on 6th day
1	Dengue Ward	72	79
2	ICU Surgical	77	86
3	ICU Surgical -from patient's bed	48	65
4	ICU Medical	73	77
5	COVID-19 Isolation Room	97	99
6	Operation Theatre (After operation)	66	79
7	Operation Theatre during Operation	86	93
8	Paediatrics	95	103
9	Waiting Area	69	92
10	ICU – PEADS	55	78
11	Emergency unit	55	65
12	Nephrology Unit	69	75
13	Thalassemia	56	59
14	Gynaecology	72	78
15	Main Entrance	86	105
	Total colonies	1076	1233

3.1. Collection of data from Hospitals

Data was collected from Liaquat University of Medical and Health Sciences Hyderabad of the patients admitted in Intensive care unit (ICU) who were diagnosed with fungal infections.

Table 9: Date from hospital

S. No:	Age/ Gender	Previous diseases	Radiological findings	Treatment	Mechanical Treatment	Duration of stay	CAPA	Status
1	16y/F	1.Infective endocarditis 2. Pulmonary hypertension	1.Bilateral peripheral patchy infiltrates	1.Moxiget 2.Omezole 3.Ceftreaxine	1.NRB 2.CPAP	15	Yes	Death
2	65y/F	1.DM 2.HTN	1.Bilateral peripheral patchy infiltrates 2.Consolidation	1.Meroneum 2.Moxi 3.Clexane 4.Decadroin 5.Anti-Fungal	Ventilator	23	YES	Death

3.2. Observations from retrospective study

- Two patients were diagnosed with Fungal infection who were associated with Diabetes mellitus and Hypertension, needed the ICU admissions.
- Only two patients died who were diagnosed with Fungus infections.
- Bilateral peripheral patchy infiltrates were common in all the patients (Patients severely infected both lungs and mostly they required ventilator).
- Diabetes mellitus and hypertension were common in all the patients.
- Both patients could not survive.

4. Discussion and Conclusion

This study examined the 15 different locations from Liaquat University of Medical and Health Sciences Hyderabad for fungal assessment by active and passive sampling, Active method showed more fungal colonies than the passive method. Fungal colonies also increased in afternoon session that could be the reason for temperature variation which encourages the maximum growth for fungal presence in outer environment. Each locations shows different fungal results. Microorganisms are present in air at a greater number and hence it was observed on main entrance gate with the most number of colonies range from (115-144). Paediatrics ward and Operation theatre had the most number of colonies in the Month of October and November, respectively and also different values were obtained at different locations.

Different parameters need to be examined such as ventilations system, people visiting hospitals, number of people present in a particular ward, temperature, weather pattern and hospital cleaning system.ICU also recorded the significant number of colonies. Fungal colonies increased on 6th day of incubation. Malt extract agar is suitable media for fungal assessment. It was observed that two patients were diagnosed with Fungal infection Patients with Diabetics and hypertension needed the ICU admissions. Only two patients died who were diagnosed with Fungus infections. Fungal assessment must be ensured in hospital environment to overcome nosocomial infections. Patients admitted in ICU are at a great risk of developing nosocomial infections. Fungal assessment is a evidence that aspergillosis infections could be a great threat in the hospitals of Hyderabad.

The microbiological quality of the air in hospitals is a significant parameter to control healthcare associated infections, and regular microbial monitoring can represent a useful tool to assess environmental quality and to identify critical situations which require corrective intervention. The microbiological content of the air can be monitored by two main methods, one active and one passive. In this study both methods were used to examine different location of hospitals by comparing the results of both the methods.

This study depicts retrospective study of Covid-19 patients to conduct fungal assessment which will help to take safety in health care systems. In the alarming situation of covid-19, microbial monitoring in hospitals and screening for covid-19 patients must be made compulsory in order to control the deadly nosocomial and pathogenic diseases. However, it was observed that no fungal screening has been carried out of covid-19 and patients who are admitted in ICU so that is the reason the cases of fungal infections remain unknown. This highlights the importance of fungal assessment, screening and diagnosis of aspergillosis. Steps must be taken to ensure safety in health care systems where deadly infections are present and limitations for indoor air quality in hospitals must be managed and diagnosis of fungal species must be ensured in hospitals especially in the screening of Covid-19 patients.

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