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Filamentous fungi associated with COVID-19 and its susceptibility to some antifungals

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Abstract---The purpose of this study was to conduct a survey of the fungal species associated with COVID-19 viral infection in 150 patients who were admitted to the intensive care unit (ICU) in Al-Diwaniyah Teaching Hospital in Al-Diwaniyah City, Iraq, for a period of five months beginning in October 2021 and ending in February 2022. The survey was to be conducted over the course of the period from October 2021 to February 2022. According to the findings, yeasts were more prevalent than any of the other detected fungal species, accounting for 98 of the total isolates (65.33 percent). While filamentous fungus accounted for 19 isolates (12.33 percent), including the predominance of *Aspergillus flavus* with 6 isolates (40 percent) in comparison to the *Aspergillus* spp. ratio, these fungi were found to be rather uncommon. In light of the fact that this publication provided evidence of the isolation of *Aspergillus sydowii* from COVID19 patients for the first time anywhere in the world: In addition, we drew attention to the outstanding activity of the antifungal medications amphotericin B, itraconazole, and voriconazole, all of which have a high susceptibility rate.

Keywords---COVID-19, yeast, filamentous fungi, antifungal, amphotericinb, itraconazole, voriconazole.

Introduction

World Health Organization (WHO) labeled the new coronavirus (COVID-19) outbreak a worldwide pandemic on March 11, 2020 (WHO, 2020). It is a respiratory condition that has a negative impact on the overall health of the individual (Singh & Singh, 2020). Fever, dry cough, weariness, dyspnea, anosmia, ageusia, or a combination of these symptoms are the most often reported clinical symptoms in patients (Huang et al., 2020). COVID-19 infection symptoms may

appear 2–14 days after exposure (based on the incubation period of COVID-19 virus). Clinical symptoms in SARS-CoV-2 infected patients are often various, ranging from no symptoms to severe sickness. These clinical symptoms can be further classified into four groups, which are as follows: asymptomatic; mild; moderate; severe; and critical illness (Raoult et al., 2020). Critically sick COVID-19 patients had increased pro-inflammatory (IL-1, IL-2, IL-6, TNF- α) and anti-inflammatory (IL-4, IL-10) cytokine levels, fewer CD4 interferon-gamma countenance, and less CD4 and CD8 cells. This acute clinical state raises the hazard of deadly fungal infections (Pemán et al., 2020).

There was an increase in the number of re-infections due to the severe immunomodulation and lymphocyte depletion generated by the virus and its treatment. Incidence and mortality of (CAPA) are on the rise (David et al., 2022). CAPA has been recorded in between 5% and 10% of severely unwell individuals with COVID-19 (coronavirus disease 2019). Hospitals' rates of occurrence range from zero to three-thirds of one percent (Clancy & Nguyen, 2022). As a consequence of the SARS-CoV-2 pandemic, mucormycosis has become a major problem for the worldwide pandemic, particularly in Asian countries (Pushparaj et al., 2022). Several hypotheses relate mucormycosis to serious COVID-19 patients who are immunocompromised and/or have concomitant co-morbidities, as an example, Covid has been linked to diabetes, which in turn has been linked to an increased risk of mucormycosis. (Bhatia, 2022). The purpose of our research is to carry out a survey of the fungal species that are linked to COVID-19 especially the filamentous fungi, and the activity of some antifungals against it.

Materials and Methods

Collection of the specimens and data

During the research period beginning in October 2021 and continuing through February 2022, a total of 150 clinical samples were collected from patients who were confirmed to be infected with COVID-19 by using PCR. These patients were hospitalized in the intensive care unit (ICU) at Al-Shifa Center of Al-Diwaniyah Teaching Hospital, which is located in the Al-Diwaniyah governorate of Iraq. Oral swabs, pharyngeal swabs, nasal swabs, and sputum samples were all included in the collection of samples. When it came to the swabs, sterile cotton swabs were rotated inside the oral cavity of the patient, and then they were stored in plastic containers until they were needed. During the process of collecting sputum samples, a container with a diameter of 5 centimeters was used. After rinsing the mouth with water to reduce the number of germs present in the mouth and to dilute the saliva, samples were obtained. It is important to avoid swallowing sputum and instead spit it out into a clean container as fast as possible.

Specimens' cultivation

For each sputum sample, 0.1 mL of the specimen was removed and streaked onto Sabouraud dextrose agar medium (Pashley et al., 2012). The swabs streaked directly onto SDA; three replications of the culture were made to ensure that the fungal growth was not contaminated during the culture process. The dishes were incubated at a temperature of 37°C for 24 hours (Atlas, 1995). Then for

subculture we used another culture media like, Czapiks dox agar, and Potato Dextrose Agar for diagnostic reasons.

Identification of Fungal species

Depending on the culture and microscopic properties of the fungus as stated in (Kidd et al., 2016).

Antifungal susceptibility test

Antifungal susceptibility testing using disk diffusion was carried out on non-supplemented MHA in accordance with the procedure outlined in the CLSI M 51-A publication (CLSI, 2010). To summarize, the mold stock inoculum suspension was applied to the whole surface of the MHA using a non-toxic cotton swab. This was done so that the mold would not be diluted. On the surface of each of the MHA plates that had been infected, disks containing antifungals were inserted. After 24 and 48 hours of incubation at 35°C, the plates were examined for their results. The CLSI M51-A standard was followed in order to interpret the zone diameter. The antifungal disks that tested are AmphotericinB (AMB) 20 µg/ml, Itraconazole (IT) 10 µg/ml, Ketoconazole (KT) 10 µg/ml, Fluconazole (FLC) 25 µg/ml, Voriconazole (VRC) 1 µg/ml. (the antifungal disks manufactured by Liophilcm, Italy)

Statistical analysis

Statistical analysis of the data was carried out using one-way ANOVA with the least significance difference (LSD) using the statistical analysis software program (Special Package for Statistical Science SPSS version 26), with a significant value $P \leq 0.05$.

Results and Discussion

Isolation and Diagnosis

A number of the agronomic and microscopic characteristics were investigated in order to demonstrate the yield of dangerous fungus isolates that were isolated from COVID19 patients. The following are some of the characteristics that were investigated:

Aspergillus spp

Cultural Characteristics

The colonies growing on Czapi's dox agar and Sabouraud Dextrose Agar (SDA) appeared in the form of Cottony fluffy to velvety white mycelium growth, shortly coated with copious amounts of spores quickly change to green, yellow, orange, black, or brown, could be seen in all of the petri dishes after three days to five days. It's possible to see different shades of the same hue at different places.

Microscopic Characteristics

In order to carry out microscopic characterization of the fungal isolate, a lactophenol cotton blue mount of the growth was prepared. This was done from the growth. They were predominantly made up of upright conidiophores. The conidiophores branched out into vesicles that were coated in phialides at their tips (biseriate).

Diagnosed isolates

An assessment of their morphology (both macroscopic and microscopic), as in Figure (4–7), was performed using a reference guide (Kidd et al., 2016; Sciortino, 2017). In order to make the best guess as to the species to which they belonged; we identified the following:

- *Aspergillus niger* (n=5) isolates, on culture medium the basal felt of the colonies is either white or yellow in color, and it is covered with a thick coating of darkish brown to black conidial heads. Under microscope the walls of conidiophore stipes are smooth and hyaline, or they begin to darken as they approach the vesicle. The conidial heads are biseriate, and the phialides are born on brown metulae that are often septate. The conidia range in size from globose to sub globose and have rough walls. Their color ranges from dark brown to black.
- *Aspergillus flavus* (n=6) isolate, on culture media yellow colonies with radial channels become brilliant to dark yellow-green with age. Under microscope some conidial heads have phialides carried directly on the vesicle (uniseriate). Near the vesicle, conidiophore stipes are hyaline and rough. Pale green, globose, echinulate conidia.
- *Aspergillus fumigatus* (n= 3) isolates, on culture media blue-green colonies have a suede-like. Microscopic examination revealed conidiophore-covered surface. Columnar conidial heads are commonly shorter and uniseriate. Short, smooth-walled conidiophore stipes with conical-shaped terminal vesicles with one row of phialides. Conidia are globose to sub globose, green, and coarsely roughened.
- *Aspergillus sydowii* (n=1) isolate. on culture media circle with brown to orange margins colonies have a suede-like The isolated fungus had conidiophores, and one could discern conidia globose in shape, metulae, and phialides.

Regarding this point, Banu et al. (2013) indicate that the *Aspergillus* colonies exhibit several phenotypic traits when grown on SDA, and this outcome is in line with what De Carolis et al. (2012) mentioned in their study that *Aspergillus* Colony morphology and microscopic features are presently used in clinical mycology laboratories to identify filamentous fungus.

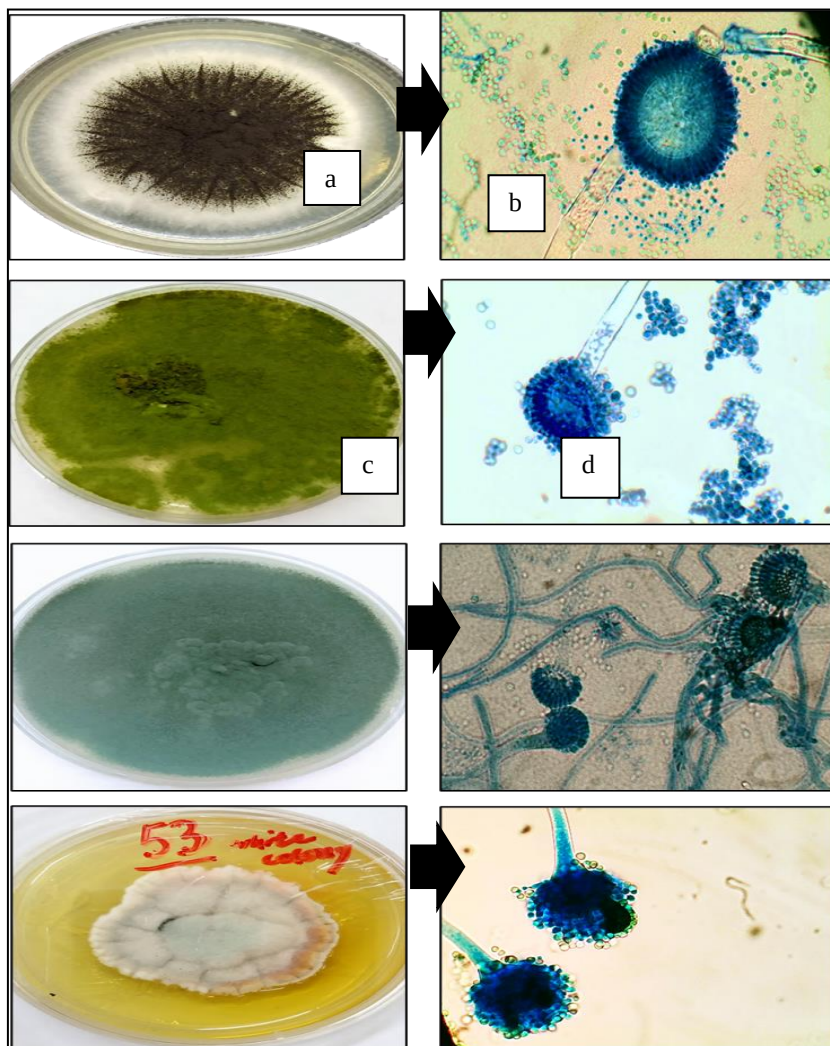


Fig.1. *Aspergillus* sp. Culture on Czapek's dox agar medium at 37°C for 5 days, and microscopic morphology (40X), a. *Aspergillus niger*, b. *Aspergillus flavus*, c. *Aspergillus fumigatus*, d. *Aspergillus sydowii*

Rhizopus oryzae **Cultural Characteristics**

The initial color of *R. oryzae* colonies is white, cottony on SDA and PDA at 37 °C, but they mature to a brownish color and rapidly can reach a thickness of around 1 centimeter. With this respect, Kwon et al. (2011) indicates that the *R. oryzae* colonies initially white and cotton-like eventually became brownish-grey to black due to the heavy accumulation of sporangia, and the mycelial growth was optimal at 30°C, although good growth was also seen at 37°C on PDA. and this result is consistent with what Manghwar et al. (2015) mentioned about the appearance of *R. oryzae* mycelium SDA was initially white but gradually turned grayish black with a web-like texture.

Microscopic Characteristics

Hyphae are hyaline, varying in size and nonseptate. Sporangiphores are dark brown, unbranched, and rhizoids are abundantly produced from the foot cells. Foot cells are often seen at the end opposite the sporangium. Sporangia are round, dark brown to grayish black, with a flattened base, and contain many spores. This finding is consistent with what was presented by Manghwar et al. (2015) in their study that sporangia, apophysis, sporangiophores, and rhizoids made up the bulk of *R. oryzae* microscopic structure.

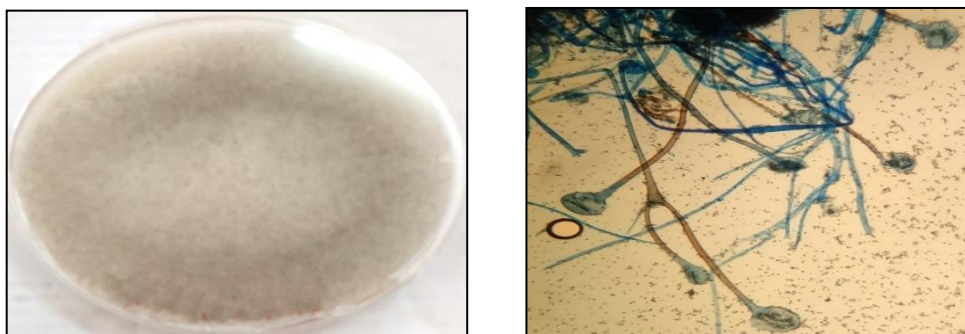


Fig.2. *R. oryzae* Culture on SDA medium at 37°C for 3 days, and microscopic morphology (40X)

Teleromyces marneffei Cultural Characteristics

A colony's color might range from green, blue, gray, white, yellow, or even pink, depending on the stage of development and the incubation period, so after three days of incubation on SDA at 25°C, the colony's surface is covered with a fine green, powdery or velvety layer, *Penicillium marneffei* that generate red pigment in the agar in the fifth day of incubation. this result is in accordance with findings reported by Sethuraman et al. (2020) who demonstrated that in 72 hours of growth on SDA at 25°C, greenish velvety mycelial colonies formed, which, after two more days of incubation, developed a diffuse red color.

Microscopic Characteristics

A mount of lactophenol cotton blue showed Phialides are generated on primary conidiophores that are short branching or unbranched. Branching can be achieved by forming metulae, which provide the impression of a brush. Chains of phialides generate conidia, which can be flat, elliptical, cylindrical, or spindle-shaped. This finding in line with Hart et al. (2012) there are septate hyphae with smooth-walled hyaline conidiophores that have bi-vertical ends with 3–5 metulae and 4–5 phialides on each.

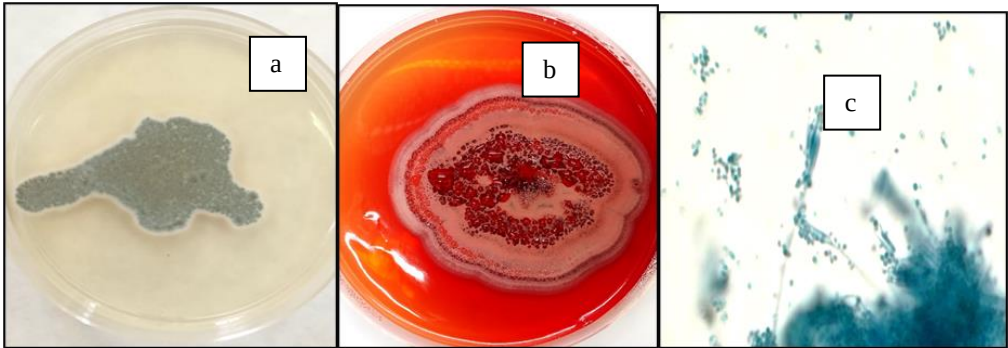


Fig.3: a) *T. marneffei* Culture on SDA medium at 25°C for 3 days, b) Culture on SDA medium at 25°C for 7days, c) microscopic morphology (40X),

Coccidioides immitis
Cultural Characteristics

C. immitis colonies start off white and may develop cottony. then become white and fluffy. As colonies age, they may become gray-white this result in line with Kantarcioglu et al. (2014) who demonstrated that in 4–5 days at 30° C and 37° C, SDA cultures developed glabrous colonies surrounding tissue biopsy specimens. After 2–3 weeks, colonies were white, floccose, and in consistent with findings by Cordeiro et al. (2006) that the *Coccidioides* colony was macromorphologically examined in PDA and SDA, revealing the apiculated, velvety, white or cream-colored

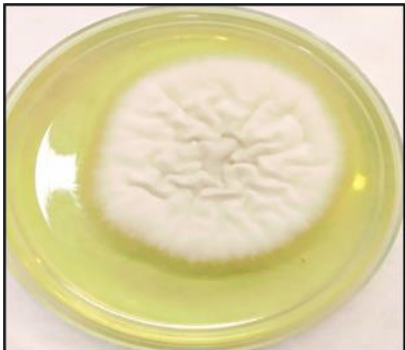


Fig.2. *C. immitis* Culture on SDA medium at 37°C for 3 days

Isolated Fungi

In the present study, it was discovered that 102 patients out of 150 had a fungal co-infection Table 1. A total of 117 distinct fungal isolates were retrieved from patients, which brings the total number of fungal isolates from this study to 117. As is evident by looking at Table 2, several of the isolates consisted of yeasts and molds:

TABLE 1: Percentage of Infection type
infection type covid19 only covid19+fungi* Total

number	48	102	150
Percentage%	32	68	100
*There are S. D. LSD= 26.172 P VALUE=0.04719			

Table 2
Fungal species isolates

Fungi Species	Number of Isolates	Percentage isolates	to 117 Samples	Percentage to 150
yeast	98	83.76		65.33
filamentous	19	16.24		12.66

There are no significant differences between fungi species

Aspergillus spp. Frequency

The recent study found that *Aspergillus* is the second prevalent fungi identified from Covid-19 patients, with a 10 % frequency. As seen in the table (1), *Aspergillus flavus* was the most common (40%) than the other isolated *Aspergillus* species (*Aspergillus niger* 33.33%, *Aspergillus fumigatus* 20% and *Aspergillus sydowii* 6.67%).

Table 1
Aspergillus spp. Isolates

<i>Aspergillus</i> sp.	isolates	Percentage to <i>Aspergillus</i> sp. Isolates
<i>Aspergillus flavus</i>	6	40
<i>Aspergillus niger</i>	5	33.33
<i>Aspergillus fumigatus</i>	3	20.00
<i>Aspergillus sydowii</i>	1	6.67
total	15	100.00

There are no significant differences between the species

In view of the information presented above on the percentage of *Aspergillus* isolates, we observe that *A. flavus* and *A. niger* are the most prevalent species. Generally, this is a sign that non-fumigatus *Aspergillus* species are in dominance. This result lends credence to the conclusions drawn by previous researchers in this field Al-Wathiqi et al. (2013); Taghizadeh-Armaki et al. (2017); Zarrinfar et al. (2012) who have found that non-fumigatus *Aspergillus* species, such as the *A. flavus*, are the most common cause of IPA in tropical and subtropical climatic zones. This finding in line with Abdalla et al. (2020) who reported that *A. terreus* or *A. niger* was suspected of causing CAPA in COVID-19 patients in Qatar, to substantiate the claim that Middle Eastern and South Asian non fumigatus *Aspergillus* species are more prevalent than in Europe and North America.

The yields and the percentage of *Aspergillus* in our study were higher compared to those of other studies by Schein et al. (2020) from France and Yang et al. (2020) from China, which reported an incident rate with 2.4% and 5.8% respectively . on the other hand, our finding is lower to what find by Rutsaert et al. (2020) from the

Netherlands and Alanio et al. (2020) from France Koehler et al. (2020) from Germany with (35%, 33%, 26.3% respectively) and indicate the prevalence of *A. fumigatus* , while Nasir et al. (2020) from Pakistan record 21.7% and *A. flavus* has the higher rate of isolation than other *Aspergillus sp.* .The mentioned studies reflect a wide-ranging incident and another indication for the variation in dominant of *Aspergillus sp.* as causing for Aspergillosis between different geographical zones. In our study we record an intriguing finding which is the first isolation globally of *Aspergillus sydowii* from COVID-19 patient based to the available literatures.

Rhizopus oryzae

According to our findings, *Rhizopus oryzae* (which causes Mucormycosis) came in at position three on the incidence scale with (1.33%) and percent of (1.71%) when compared to the remaining fungal isolates that were recovered and diagnosed as having originated from COVID-19 patients, as shown in table (2).

Table 2
Rhizopus sp. Isolates

<i>Rhizopus sp.</i>	isolates	Percentage to <i>Rhizopus sp.</i> Isolates	Percentage to fungal isolates (117)
<i>Rhizopus oryzae</i>	2	100	1.71

This pattern of results is consistent with the finding of Masci and Wormser (2005); Nazir et al. (2021) that *Rhizopus Oryzae* strains account for sixty percent of the cases of human mucormycosis, and *Rhizopus Oryzae* makes up ninety percent of rhino orbital brain (ROCM), this is consistent with A meta-analysis of 851 instances of mucormycosis by Jeong et al. (2019), which indicated that the species *Rhizopus* was the most prevalent one to be isolated. And in consistent with Prakash et al. (2019) work that deals with mucormycosis at India's four largest tertiary care facilities, they found that *Rhizopus oryzae* (n = 124, 51.9 percent) was the major agent found, followed by *Rhizopus microsporus* (n = 30, 12.6 percent), *Apophysomyces variabilis* (n = 22, 9.2 percent) and *Rhizopus homothallicus* (n = 6, 2.5 percent).

Also , we isolate *Rhizopus Oryzae* from a nasal swab , which indicate the colonization of this genus to the nasal sinuses , this finding in line with Uddin (2021) who point out that nose , sinuses are the mostly affected parts of the body. Satish et al. (2021) have stated that Mucormycosis linked to COVID-19 has been identified in both Europe and the United States (CAM). According to reports from Iran, Pakistan, Bangladesh, and Iraq (and other countries), CAM instances have been reported.

Talaromyces marneffe

According to our findings, *Talaromyces marneffe* (which causes Talaromycosis) on the incidence scale with (0.67%) and a percent of (0.85%) when compared to the remaining fungal isolates that were recovered and diagnosed as having originated from COVID-19 patients, as shown in table (3).

Table 3
Talaromyces sp. Isolates

<i>Talaromyces sp.</i>	isolates	Percentage to <i>Talaromyces sp.</i> Isolates	Percentage to fungal isolates (117)
<i>Talaromyces marneffe</i>	1	100	0.85

(Narayanasamy et al., 2021) Death and morbidity rates from acute and chronic pulmonary mycoses, notably endemic diseases like Talaromycosis, remain alarmingly high despite breakthroughs in antifungal therapy (Maitre et al., 2021).

Coccidioides immitis

According to our findings, *Coccidioides immitis* (which causes Coccidiomycosis) on the incidence scale with (0.67%) and a percent of (0.85%) when compared to the remaining fungal isolates that were recovered and diagnosed as having originated from COVID-19 patients, as shown in table (4).

Table 4
Coccidioides sp. Isolates

<i>Coccidioides sp.</i>	isolates	Percentage to <i>Coccidioides sp.</i> Isolates	Percentage to fungal isolates (117)
<i>Coccidioides immitis</i>	1	100	0.85

Consistent findings with Shah et al. (2020) who gave a description of the first instance of Coccidiomycosis coinfection with CoV-2 that has ever been documented, this is in line with Chang et al. (2020) who report identification of Coccidiomycosis with COVID19 in a female (48 y) and stated the health care system is on the verge of collapsing under the strain of the COVID-19 pandemic, which is why screening, triaging, and treatment efforts need to be stepped up immediately. It is simple to forget about endemic illnesses like *Coccidioides immitis* or to incorrectly believe that they are part of a pandemic.

Antifungal susceptibility test

Table 5
Antifungal susceptibility for filamentous fungi (disc diffusion)

Fungi. (n)	Sensitivity	Antifungal agents				
		AMB 20µg/ml	FLC 25µg/ml	KT 10µg/ml	IT 10µg/ml	VRC 1µg/ml
<i>Aspergillus flavus</i> (6)	S	6	4	3	6	6
	S-DD					
	R		2	3		
<i>Aspergillus fumigatus</i> (3)	S	3	2	2	3	3
	S-DD					
	R		1	1		
<i>Aspergillus niger</i> (5)	S	5	4	3	5	5
	S-DD					
	R		1	2		
<i>Aspergillus sydowii</i> (1)	S	1	1	1	1	1
	S-DD					
	R					
<i>Coccidioides immitis</i> (1)	S	1		1	1	1
	S-DD					
	R		1			
<i>Talaromyces marneffei</i> (1)	S	1		1	1	1
	S-DD					
	R		1			
<i>Rhizopus oryzae</i> (2)	S	2	2	2	2	2
	S-DD					
	R					
Total` 19	S (%)	100	14 73.7	13 68.4	100	100
	S-DD					
	R (%)		5 26.3	6 31.6		

It is clear from observing the table (5) that the antifungal that was tested had a definite degree of sensitivity when put up against the filamentous fungus. AmB, IT, VRC obtained sensitivity with 100%, while FLC, KT get an obvious resistance with (26.3, 31.6) % respectively. These results in accordance with Kumar et al. (2010) who reported that the disc diffusion technique was used for measuring the susceptibility of antifungals toward *Aspergillus flavus*, and the results indicated that not a single strain was fluconazole sensitive. In addition, ketoconazole demonstrated a sensitivity of sixty percent at Against itraconazole, 100 percent strains were sensitive. Amphotericin B had a 96 percent inhibition rate. The findings of the study by Thompson et al. (2017), which are consistent with our findings, indicate that *Coccidioides immitis* has evolved a resistance to the antifungal medication fluconazole. Our findings for *T. marneffei* are consistent with those of Lei et al. (2018) who stated that their study results of antifungals against *T. marneffei* support the use of Amphotericin B, Itraconazole, Voriconazole, and Posaconazole in the clinical care of Talaromycosis but raise concerns about fluconazole resistance.

Conclusion

Fungal co-infection poses a significant health risk in patients infected with coronavirus (COVID-19). *Aspergillus* sp. that causes Aspergillosis and *Rhizopus* sp., which causes Mucormycosis, is becoming increasingly common as the globe

continues to fight COVID-19 due to immunological issues that weaken the defenses of the body against opportunistic and true pathogenic fungi. Furthermore, the antifungals AmphotericinB, Itraconazole, and Voriconazole have good activity with a high susceptibility ratio. Lastly, this study found that COVID-19 is linked to a high number of fungal infections. Because of this, patients with COVID-19 should be tested for fungal infections as soon as possible to reduce the chance of getting a more serious illness. This is because early detection is key to treating fungal co-infections.

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References

- Abdalla, S., Almaslamani, M. A., Hashim, S. M., Ibrahim, A. S., & Omrani, A. S. (2020). Fatal Coronavirus Disease 2019-associated Pulmonary Aspergillosis; A Report of Two Cases and Review of the Literature. *IDCases*, 22, e00935-e00935. <https://doi.org/10.1016/j.idcr.2020.e00935>
- Al-Wathiqi, F., Ahmad, S., & Khan, Z. (2013). Molecular identification and antifungal susceptibility profile of *Aspergillus flavus* isolates recovered from clinical specimens in Kuwait. *BMC Infectious Diseases*, 13(1). <https://doi.org/10.1186/1471-2334-13-126>
- Alanio, A., Delli re, S., Fodil, S., Bretagne, S., & M garbane, B. (2020). Prevalence of putative invasive pulmonary aspergillosis in critically ill patients with COVID-19. *The Lancet. Respiratory medicine*, 8(6), e48-e49. [https://doi.org/10.1016/S2213-2600\(20\)30237-X](https://doi.org/10.1016/S2213-2600(20)30237-X)
- Atlas, R. M. (1995). Principles of Microbiology. Mosby-Year Book. Inc., St-Louis, USA.
- Banu, A., Anand, M., & Eswari, L. (2013). A rare case of onychomycosis in all 10 fingers of an immunocompetent patient. *Indian Dermatology Online Journal*, 4(4). <https://doi.org/10.4103/2229-5178.120649>
- Bhatia, M. (2022). The rise of mucormycosis in Covid-19 patients in India. *Expert Review of Anti-infective Therapy*, 20(2), 137-138. <https://doi.org/10.1080/14787210.2021.1960822>
- Chang, C. C., Senining, R., Kim, J., & Goyal, R. (2020). An Acute Pulmonary Coccidioidomycosis Coinfection in a Patient Presenting With Multifocal Pneumonia With COVID-19. *Journal of Investigative Medicine High Impact Case Reports*, 8, 2324709620972244. <https://doi.org/10.1177/2324709620972244>
- Clancy, C. J., & Nguyen, M. H. (2022). Coronavirus Disease 2019-Associated Pulmonary Aspergillosis: Reframing the Debate. *Open Forum Infect Dis*, 9(5), ofac081. <https://doi.org/10.1093/ofid/ofac081>
- CLSI. (2010). Performance Standards for Antifungal Disk Diffusion Susceptibility Testing of Filamentous Fungi; Informational Supplement CLSI document M51-S1. In: Clinical and Laboratory Standards Institute Wayne.
- Cordeiro, R. A., Brilhante, R. S. N., Rocha, M. F. G., Fechine, M. A. B., Camara, L. M. C., Camargo, Z. P., & Sidrim, J. J. C. (2006). Phenotypic characterization and ecological features of *Coccidioides* spp. from Northeast Brazil. *Medical mycology*, 44(7), 631-639. <https://doi.org/10.1080/13693780600876546>

- David, F., Morais, J. R., Beires, F., Greenfield, H., & Fernandes, G. L. (2022). Invasive Pulmonary Aspergillosis after COVID-19 Pneumonia. *Eur J Case Rep Intern Med*, 9(3), 003209. https://doi.org/10.12890/2022_003209
- De Carolis, E., Posteraro, B., Lass-Flörl, C., Vella, A., Florio, A. R., Torelli, R., Girmenia, C., Colozza, C., Tortorano, A. M., Sanguinetti, M., & Fadda, G. (2012). Species identification of *Aspergillus*, *Fusarium* and *Mucorales* with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clinical Microbiology and Infection*, 18(5), 475-484. <https://doi.org/10.1111/j.1469-0691.2011.03599.x>
- Hart, J., Dyer, J. R., Clark, B. M., McLellan, D. G., Perera, S., & Ferrari, P. (2012). Travel-related disseminated *Penicillium marneffei* infection in a renal transplant patient. *Transplant Infectious Disease*, 14(4), 434-439. <https://doi.org/10.1111/j.1399-3062.2011.00700.x>
- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., & Gu, X. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, 395(10223), 497-506.
- Jeong, W., Keighley, C., Wolfe, R., Lee, W. L., Slavin, M. A., Kong, D. C. M., & Chen, S. C. (2019). The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. *Clin Microbiol Infect*, 25(1), 26-34. <https://doi.org/10.1016/j.cmi.2018.07.011>
- Kantarcioglu, A. S., Sandoval-Denis, M., Aygun, G., Kiraz, N., Akman, C., Apaydin, H., Karaman, E., Guarro, J., de Hoog, G. S., & Gurel, M. S. (2014). First imported coccidioidomycosis in Turkey: A potential health risk for laboratory workers outside endemic areas. *Medical mycology case reports*, 3, 20-25. <https://pubmed.ncbi.nlm.nih.gov/24567896>
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3930961/>
- Kidd, S., Halliday, C. L., Alexiou, H., & Ellis, D. H. (2016). *Descriptions of Medical Fungi*. CutCut Digital. <https://books.google.iq/books?id=G95gjwEACAAJ>
- Koehler, P., Cornely, O. A., Böttiger, B. W., Duse, F., Eichenauer, D. A., Fuchs, F., Hallek, M., Jung, N., Klein, F., & Persigehl, T. (2020). COVID-19 associated pulmonary aspergillosis. *Mycoses*, 63(6), 528-534.
- Kumar, R., Shrivastava, S. K., & Chakraborti, A. (2010). Comparison of Broth Dilution and Disc Diffusion Method for the Antifungal Susceptibility Testing of *Aspergillus flavus*. *American Journal of Biomedical Sciences*, 202-208. <https://doi.org/10.5099/aj100300202>
- Kwon, J.-H., Kim, J., & Kim, W.-I. (2011). First Report of *Rhizopus oryzae* as a Postharvest Pathogen of Apple in Korea. *Mycobiology*, 39(2).
- Lei, H. L., Li, L. H., Chen, W. S., Song, W. N., He, Y., Hu, F. Y., Chen, X. J., Cai, W. P., & Tang, X. P. (2018). Susceptibility profile of echinocandins, azoles and amphotericin B against yeast phase of *Talaromyces marneffei* isolated from HIV-infected patients in Guangdong, China. *European Journal of Clinical Microbiology & Infectious Diseases*, 37(6), 1099-1102. <https://doi.org/10.1007/s10096-018-3222-x>
- Maitre, T., Cottenet, J., Godet, C., Roussot, A., Abdoul Carime, N., Ok, V., Parrot, A., Bonniaud, P., Quantin, C., & Cadranel, J. (2021). Chronic Pulmonary Aspergillosis: Prevalence, favouring pulmonary diseases and prognosis. *European Respiratory Journal*, 2003345. <https://doi.org/10.1183/13993003.03345-2020>

- Manghwar, H., Naz, S., Gul, S., Chaudhary, H., & Munis, M. F. H. (2015). First report of *Rhizopus oryzae* causing fruit rot of citrus medica L. in Pakistan. *JOURNAL OF PLANT PATHOLOGY*, 93, 209-220.
- Maschi, J. R., & Wormser, G. P. (2005). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 6th Edition Edited by Gerald L. Mandell, John E. Bennett, and Raphael Dolin Philadelphia: Elsevier Churchill Livingstone, 2005. 3661 pp., illustrated. \$329 (cloth). *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, 41(2), 277-277. <https://doi.org/10.1086/431221>
- Narayanasamy, S., Dougherty, J., van Doorn, H. R., & Le, T. (2021). Pulmonary Talaromycosis: A Window into the Immunopathogenesis of an Endemic Mycosis. *Mycopathologia*, 186(5), 707-715. <https://doi.org/10.1007/s11046-021-00570-0>
- Nasir, N., Farooqi, J., Mahmood, S. F., & Jabeen, K. (2020). COVID-19-associated pulmonary aspergillosis (CAPA) in patients admitted with severe COVID-19 pneumonia: an observational study from Pakistan. *Mycoses*, 63(8), 766-770.
- Nazir, S., Khan, A., Noor, A., Rehman, R., & Khalid, B. (2021). Mucormycoses in Covid-19 Patients. *Pakistan Journal of Medical and Health Sciences*, 15(9), 2837-2840. <https://doi.org/10.53350/pjmhs211592837>
- Pashley, C. H., Fairs, A., Morley, J. P., Taylor, S., Agbetile, J., Bafadhel, M., Brightling, C. E., & Wardlaw, A. J. (2012). Routine processing procedures for isolating filamentous fungi from respiratory sputum samples may underestimate fungal prevalence. *Medical mycology*, 50(4), 433-438.
- Pemán, J., Ruiz-Gaitán, A., García-Vidal, C., Salavert, M., Ramírez, P., Puchades, F., García-Hita, M., Alastruey-Izquierdo, A., & Quindós, G. (2020). Fungal co-infection in COVID-19 patients: Should we be concerned? *Revista Iberoamericana de Micología*, 37(2), 41-46. <https://doi.org/https://doi.org/10.1016/j.riam.2020.07.001>
- Prakash, H., Ghosh, A. K., Rudramurthy, S. M., Singh, P., Xess, I., Savio, J., Pamidimukkala, U., Jillwin, J., Varma, S., Das, A., Panda, N. K., Singh, S., Bal, A., & Chakrabarti, A. (2019). A prospective multicenter study on mucormycosis in India: Epidemiology, diagnosis, and treatment. *Medical mycology*, 57(4), 395-402. <https://doi.org/10.1093/mmy/myy060>
- Pushparaj, K., Kuchi Bhotla, H., Arumugam, V. A., Pappusamy, M., Easwaran, M., Liu, W. C., Issara, U., Rengasamy, K. R. R., Meyyazhagan, A., & Balasubramanian, B. (2022). Mucormycosis (black fungus) ensuing COVID-19 and comorbidity meets - Magnifying global pandemic griever and catastrophe begins. *Sci Total Environ*, 805, 150355. <https://doi.org/10.1016/j.scitotenv.2021.150355>
- Raoult, D., Zumla, A., Locatelli, F., Ippolito, G., & Kroemer, G. (2020). Coronavirus infections: Epidemiological, clinical and immunological features and hypotheses. *Cell stress*, 4(4), 66.
- Rutsaert, L., Steinfert, N., Van Hunsel, T., Bomans, P., Naesens, R., Mertes, H., Dits, H., & Van Regenmortel, N. (2020). COVID-19-associated invasive pulmonary aspergillosis. *Ann Intensive Care*, 10(1), 71. <https://doi.org/10.1186/s13613-020-00686-4>
- Satish, D., Joy, D., & Ross, A. B. (2021). Mucormycosis co-infection associated with global COVID-19: A case series from India. *Int. J. Otorhinolaryngol. Head Neck Surg*, 7, 815-820.

- Schein, F., Munoz-Pons, H., Mahinc, C., Grange, R., Cathébras, P., & Flori, P. (2020). Fatal aspergillosis complicating severe SARS-CoV-2 infection: A case report. *Journal de mycologie medicale*, 30(4), 101039. <https://doi.org/10.1016/j.mycmed.2020.101039>
- Sethuraman, N., Thirunarayan, M. A., Gopalakrishnan, R., Rudramurthy, S., Ramasubramanian, V., & Parameswaran, A. (2020). *Talaromyces marneffei* Outside Endemic Areas in India: an Emerging Infection with Atypical Clinical Presentations and Review of Published Reports from India. *Mycopathologia*.
- Shah, A. S., Heidari, A., Civelli, V. F., Sharma, R., Clark, C. S., Munoz, A. D., Ragland, A. S., & Johnson, R. H. (2020). The Coincidence of 2 Epidemics, Coccidioidomycosis and SARS-CoV-2: A Case Report. *Journal of Investigative Medicine High Impact Case Reports*, 8, 2324709620930540. <https://doi.org/10.1177/2324709620930540>
- Singh, J., & Singh, J. (2020). COVID-19 and its impact on society. *Electronic Research Journal of Social Sciences and Humanities*, 2.
- Taghizadeh-Armaki, M., Hedayati, M. T., Moqarabzadeh, V., Ansari, S., Mahdavi Omran, S., Zarrinfar, H., Saber, S., Verweij, P. E., Denning, D. W., & Seyedmousavi, S. (2017). Effect of involved *Aspergillus* species on galactomannan in bronchoalveolar lavage of patients with invasive aspergillosis. *J Med Microbiol*, 66(7), 898-904. <https://doi.org/10.1099/jmm.0.000512>
- Thompson, G. R., 3rd, Barker, B. M., & Wiederhold, N. P. (2017). Large-Scale Evaluation of In Vitro Amphotericin B, Triazole, and Echinocandin Activity against *Coccidioides* Species from U.S. Institutions. *Antimicrobial agents and chemotherapy*, 61(4), e02634-02616. <https://doi.org/10.1128/AAC.02634-16>
- Uddin, K. N. (2021). Black fungus: a new threat. *BIRDEM Medical Journal*, 11(3), 164-165.
- WHO. (2020). World Health Organization Director-General opening remarks at the media briefing on COVID-19-11 March 2020. In: Geneva, Switzerland.
- Yang, X., Yu, Y., Xu, J., Shu, H., Liu, H., Wu, Y., Zhang, L., Yu, Z., Fang, M., & Yu, T. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *The Lancet respiratory medicine*, 8(5), 475-481.
- Zarrinfar, H., Saber, S., Kordbacheh, P., Makimura, K., Fata, A., Geramishoar, M., & Mirhendi, H. (2012). Mycological Microscopic and Culture Examination of 400 Bronchoalveolar Lavage (BAL) Samples. *Iranian journal of public health*, 41(7), 70-76. <https://pubmed.ncbi.nlm.nih.gov/23113213>
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3469012/>
- Rinartha, K., & Suryasa, W. (2017). Comparative study for better result on query suggestion of article searching with MySQL pattern matching and Jaccard similarity. In *2017 5th International Conference on Cyber and IT Service Management (CITSM)* (pp. 1-4). IEEE.
- Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, 29(3), 550-561. <https://doi.org/10.1002/cae.22202>