



RESEARCH ARTICLE

ROLE OF FUNGI IN COVID-19 PATIENTS WITH CHRONIC LUNG DISEASE IN RANI DURGAVATI MEDICAL COLLEGE, BANDA (FORMERLY GOVERNMENT ALLOPATHIC MEDICAL COLLEGE, BANDA 210001 (UP) INDIA.)

Mr. Dharendra Kumar Singh¹, Dr. Rajesh Sharma², Dr. Brajesh Ranjan³, Dr. Rajiv Srivastava⁴ and Dr. Sanjay Kumar Sharma⁵

1. QC manager, Cliantha Research, Noida 63.
2. Assistant Professor, Department of Community Medicine, GS Medical College & Hospital, Pilkhuwa, Hapur.
3. Associate Professor, Department of Anatomy, Rani Durgavati Medical College & Hospital, Banda.
4. Associate Professor, Department of Community Medicine, RG Medical College, Hathras (UP).
5. Professor & Head, Department of Microbiology, UNS ASMC Jaunpur.

Manuscript Info

Manuscript History

Received: 30 July 2024

Final Accepted: 31 August 2024

Published: September 2024

Key words:-

CLD, Chronic Obstructive Pulmonary Disease, Chronic Bronchitis, Bronchiectasis, Choric Asthmatics

Abstract

Background: Fungal infections of the lung have gained increasing recognition in the field of infectious disease and they are difficult to diagnosis. Advances in modern treatment like antibiotics corticosteroids, Immunosuppressants and anticancer drugs have lead to increase in opportunistic fungal infections including bronchopulmonary Candidiasis and Bronchopulmonary aspergillosis has also increased, commonly occurs in COVID-19 patients of pre existing bronchial asthma. In India there is high prevalence of chronic lung diseases including bronchial Asthma, yet the diagnosis of aspergillosis and Candidiasis. An association between asthma and aspergillosis was noted and confirmed by isolation of Aspergillus species more commonly in sputum of Asthma patients (Lum et al., 2020; Singla, N. et al., 2019).

Subject and Methods: To find out the role of fungi in 50 Corona virus (RTPCR test) positive patients of chronic lung disease (non tubercular) like COPD, Chronic Bronchitis with or without emphysema, Broncheictasis, Bronchial Asthma were included. Patients were attended and admitted in Rani Durgavati Medical College (RDMC) and Hospital, Banda (UP) India, in a one year period was taken up from December 2020 till December 2021. The diagnosis of these cases v/as established by clinical features, radiological examination of chest, ECG, pulmonary function tests. Laboratory procedure for isolation and identification of fungi in bronchoalveoiar lavage. Each specimen of Bronchoalveolar Lavage (BAL) was subjected to various laboratory procedures for isolation and identification of fungi.

Result: Different fungal species were cultured in 26% total cases. 20% isolates were positive for fungi alone whereas rest 6% cases showed both bacterial and fungal pathogens amongst fungi Candida was the predominant isolate in 16% cases followed by Aspergillus in 8% cases and Histoplasma capsulatum in just 1(2%) case. Fungal isolates in bronchial aspirates were sensitive to Amphotericin B, Fluconazole, etc.

Conclusion: COVID-19 Patients which are already containing chronic lung diseases especially COPD, bronchiectasis is more common in elderly males. The Bronchiectatic patients are also at risk of getting, fungal infections Patients of chronic lung disease who are on long-term antibiotic therapy, and or steroids are more predisposed to get these opportunistic infections. Another conclusion from it is clear that there is great need better and judicious use of antibiotics in patients to prevent drug resistance, to prevent further progression of the diseases.

Copyright, IJAR, 2024., All rights reserved.

Introduction:-

Fungal infections of the lung have gained increasing recognition in the field of infectious disease and they are difficult to diagnosis (Russo et al., 2020; Spinello et al., 2018). Advances in modern treatment like antibiotics corticosteroids, Immunosuppressants and anticancer drugs have lead to increase in opportunistic fungal infections including Bronchopulmonary aspergillosis (Azar et al.,2017; Martin et al., 2022; Li JX et al., 2017).

In India there is high prevalence of chronic lung diseases including bronchial Asthma, yet the diagnosis of aspergillosis and Candidiasis is usually not made due to lack of mycological tests in routine diagnostic laboratories. An association between asthma and aspergillosis was noted and confirmed by isolation of Aspergillus species more commonly in sputum of Asthma patients (Lum et al., 2020; Singla, N. et al., 2019). Allergic bronchopulmonary aspergillosis commonly occurs in patients of pre existing bronchial asthma it is hypersensitivity diseases caused by Aspergillus fumigatus. Sensitisation to Aspergillus antigens may occur in asthmatics without full-blown picture of ABPA (Singla, N. et al., 2019; Sanchez et al., 2009). Among fungi Candida species are common human saprophytes. The incidence of bronchopulmonary Candidiasis has also increased due to use of antibiotics and corticosteroids in patients of chronic lung disease (Martin et al., 2022; Li JX et al., 2017; Sanchez et al., 2009; Mansour et al., 2019; Y. Zuo et al., 2020; Hamdy et al., 2020).

Laboratory Diagnosis of Pulmonary Mycosis

A definitive isolation of pulmonary mycosis depends on repeated isolation of the etiological agent in culture or demonstration of specific immunological changes in the patient. The first criteria is specific but not always advisable due to faulty collection and processing of clinical specimens, paucity of the fungi elements in the material cultured, rapid loss in their viability or inadequate laboratory follow up.

Diagnosis can also be made by microscopic examination of appropriately stained tissue section and smears or wet mount of pathological specimen considering that prognosis in systemic mycosis is largely determined by administration of specific antifungal chemotherapy in early stages of disease the rapid and precise diagnosis is important for diagnosing bronchopulmonary mycosis when clinical and radiological picture is suggestive following specimens can be collected (i) Sputum (ii) Bronchial aspirate (iii) Lung biopsy (iv) Aspiration of pus from cavity or empyema (v) blood / serum for immunological studies. Also the skin sensitivity tests may be used for diagnosis (Russo et al., 2020; Spinello et al., 2018).

Subject and Methods:-

Study Design

To find out the role of fungi in COVID-19 patients of chronic lung disease in Rani Durgavati Medical College and Hospital, Banda (UP) India, from December 2020 till December 2021.

Population and Sample

In present study, 50 patients who were positive for corona virus (RTPCR test) and also associated with chronic lung diseases (non tubercular) like chronic obstructive pulmonary disease (COPD), Chronic Bronchitis with or without emphysema, Bronchiectasis, Bronchial Asthma were included. They were attended and admitted in Rani Durgavati Medical College and Hospital, Banda (UP) India, in a one year period was taken up. The diagnosis of these cases v/as established by clinical features, radiological examination of chest, ECG, pulmonary function tests.

Criteria for Selection of Patients

All COVID-19 patients who had history consistent with disease i.e. cough and expectoration on most of days during at least 3 months of the year for 2 or more consecutive years. The patients were in a state of acute exacerbation defined as any combination of increased quantity /purulence of sputum with cough dyspnoea, fever or general malaise. Patients were not on antibiotic, therapy, antifungal drugs and other drugs like corticosteroids, insulin, and immunosuppressive drugs. Asthmatics were taking only inhaled corticosteroids. Patients did not have impaired glucose tolerance test, and uraemia.

Steps of Research**Laboratory procedure for isolation and identification of fungi in bronchoalveolar lavage**

Each specimen of Bronchoalveolar Lavage (BAL) was subjected to various laboratory procedures for isolation and identification of fungi.

1). Direct Microscopy:

The Bronchoalveolar lavage was homogenised with help of a magnetic stirrer using sterile iron nails. This homogenised specimen was subjected to direct microscopy by using KOH mount and Lacto Phenol Cotton Blue (LPCB) mount.

Procedure:

One drop of 20% KOH and one drop of LPCB was taken at two ends of a clean and sterile glass slide. Then a loopful of BAL was emulsified in KOH and the other in one drop of LPCB. Separate cover slips were placed on each of the drops and examined after 20 minutes under the microscope to see the fungal elements like, hyphae, fungal heads and spores.

2). Culture:

Culture for isolation and identification of fungi, the BAL specimen was inoculated in two sets of culture media i.e Saborauds dextrose agar (SDA) plain and Sabouraud's dextrose agar with chloramphenicol (50 mg/l).

One set of the SDA culture tube1 was incubated at 37°C and another set were incubated at 25°C in a BOD incubator. All the culture media were examined for fungal growth daily during the first

Week and thereafter every 2-3 days upto 3 week before reporting the specimen as negative for fungus. All the fungal isolates were identified according to standard methods described by Julia et al., (2020); Karahalil et al., (2018); Mackie and MC Carteny 14th Edition 1996.

3). Tease Mount preparation:

Tease mount preparation of the fungus colony was made in lacto phenol cotton blue (LPCB).

Procedure:

One drop of LPCB was placed on a clean sterile slide and a small portion of the colony was removed from the culture medium with the help of a L- wire and placed in LPCB stain. The mycelial mass of the colony was teased apart gently with the help of dissecting needles. It was then covered with a cover slip pressed slightly and examined under the microscope.

4). Identification of the Filamentous fungi:

Identification of the Filamentous fungi was used for identification of filamentous fungi by studying the exact morphology of the fungus (Karahalil et al., 2018).

5). Microslide Culture:

The slide culture set consists of sterilized petridish, bent glass rod, glass slide, coverslip and filter paper. The slide culture method was performed in the following way, as advocated by Procop & Roberts in the Bailey & Scott's Diagnostic Microbiology, 10th edition 1998.

A small block of Sabourauds Dextrose Agar medium of approximately 2 mm was cut using a sterile scalpel blade. A piece of filter paper was placed into a sterile culture dish. A V-shaped bent rod was positioned on it and a sterile microscopic slide was put on it. The block of agar was scooped out using the tip of a scalpel blade and was placed on the slide. With a right angled wire, the four quadrants of the agar were inoculated with the fungal colony. Finally

a sterile coverslip was applied into the surface of the agar block, the filter paper was moistened with sterile water, and the culture dish was covered with its lid and was incubated at 25°C. On appearance of satisfactory growth, the cover slip was removed and placed on a microscopic slide containing a drop of lactophenol cotton blue. The remaining agar block was then removed, a drop of lactophenol cotton blue was placed on the area of growth and a cover slip was positioned into place. The two preparations were observed microscopically to see the characteristic morphology of the fungus. Final reporting was made after correlating the findings of direct microscopy, colony characteristics and microscopic examination of tease mount preparation.

6). Characterisation of the fungus:

The fungal isolates were characterised according to:

The colony morphology on Sabourauds Dextrose Agar. By making tease mount preparation and identifying the morphology of conidiophores and fungal heads. By slide culture technique when ever required.

5. Study Instruments

The instrument used in this study was for isolation and identification of fungi in bronchoalveolar lavage.

6. Data analysis:

Automated data analysis were performed using a Microsoft Excel based invader data analysis worksheet (IDAW) developed and produced by TWT. The data was entered and analyzed through the SPSS version 10.0 (SPSS Inc, Chicago, US). Descriptive statistics was used to summarize the continuous and categorical data. Results were expressed as mean \pm standard deviation (SD), frequencies and percentages.

Observations & Result:-

This study covered 50 patients of chronic lung disease. These included patients of chronic bronchitis, chronic bronchitis with emphysema, chronic asthmatics and patients of Bronchiectasis.

Age and Sex Distribution of Chronic Lung Diseases;

Table 1 shows age and sex distribution of patients in various age groups.

Table 1:- Age And Sex Distribution Of Chronic Lung Disease Cases.

Age groups (in years)	No. of patents	Males	Female
30-40	05 (10%)	04 (08%)	01(01%)
41-50	15 (30%)	10 (20%)	05 (10%)
51-60	20 (40%)	12 (24%)	08 (16%)
61-70	10 (20%)	08 (16%)	02 (04%)
Total	50 (100%)	34 (68%)	16 (32%)

The maximum numbers of patients were in the age group of 51- 60 years followed by patients in the age group of 41-50 years.

Table 2:- Percentage of Case According To Their Age And Time Of Onset Of Diseases.

Age Groups (In Years)	Percentage (%)
41-50	40
51-60	30

The percentage of fifty patients of COAD according to their age and time of onset of disease is depicted in Table 2. The mean age of onset of disease in patients in this study was 46.9 years. From the table, maximum number of patients (40%) had their onset of disease between the age of 41-50 years while another 30% had onset of disease between the age of 51 and 60 years.

Table 3:- Distribution Of Cases According To Duration Of Illness.

Duration (in years)	No. of patients	Percentage (%)
0-3	10	20
4-6	25	50

7-10	15	30
>10	00	00
Total	50	100

The mean duration of illness in these patients was 5.35 years \pm 2.67 years Table 3 depicts the duration of illness in the patients.

Presenting Features Of Disease

All (100%) patients had cough with expectoration as their presenting symptom followed by 96% Breathlessness / Dyspnoea and only 20% had Congestive Heart Failure as presenting feature however cough with expectoration was present in varying degrees in all the patients at the time of entry in the study (Table 4).

Table 4:- Presenting Features Of Disease

Symptoms	No. of patients	Percentage
Cough with expectoration	50	100
Dyspnoea (Breathlessness)	48	96
Congestive Heart Failure	10	20

Table 5:- Different Isolates In Sputum (N = 50).

Type of Org.	Isolates	Total Number	% Age	
FUNGAL PATHOGENS				(20+06) 26% Age
	Candida albicans	3	6	
	Candida tropicalis	1	2	
	Candida parapsilosis	1	2	
	Geotrichum candidum	1	2	
	Aspergillus flavus	2	4	
	Aspergillus fumigatus	1	2	
	Asperg terreus	1	2	
Total		10	20	
BACTERIAL* FUNGAL PATHOGENS				
	Ecoli +Histoplasma capsulatum	1	2	
	E coli + Candida albicans	2	4	
Total		3	6	

Out of total 50, 10 (20%) of sputum samples showed fungal pathogens as the main isolate. Different fungal species were cultured in 26% total cases. 20% isolates were positive for fungi alone whereas in rest 6% cases both bacterial and fungal pathogens were present. Amongst fungi Candida was the predominant isolate in 16% cases followed by Aspergillus sp in 8% cases and Histoplasma, capsulatum in just 1 (2%) cases.

Table 6:- Different Isolates In Bronchial Aspirate (N = 50).

Type of Org.	Isolates	Total Number	% age	
BACTERIAL + FUNGAL PATHOGENS				(18+08) 26% Age
	E coli + candida albicans	2	4	
	Pseudomonas+candida tropicalis	1	2	
	Pseudomonas+candida parapsilosis	1	2	
	Pseudomonas+Aspergillus	4	8	

	species		
	Ecoli + Histoplasma capsulatum	1	2
Total		9	18
FUNGAL PATHOGENS ONLY			
	Geotrichum candidum	1	2
	Candida albicans	3	6
Total		4	8

Bronchial aspirates showed Different fungal species were cultured in 26% total cases. 08% isolates were positive for fungi alone whereas in rest 18% cases both bacterial and fungal pathogens were present. Amongst fungi Candida was the predominant isolate in 16% cases followed by Aspergillus sp in 8% cases and Histoplasma, capsulatum in just 1 (2%) cases.

Discussion:-

This study also reported isolates of fungi (Candida species, Aspergillus & Histoplasma species) in patients of bronchiectasis along with isolation of bacteria. In the past decades fungi have assumed a greater role in respiratory and systemic diseases, increasing use of antibiotics, corticosteroids, immunosuppressants and anticancer drugs have caused a rise in opportunistic fungal infections including Bronchopulmonary Aspergillosis. Despite high prevalence of chronic lung diseases in our country the exact incidence of bronchopulmonary aspergillosis is not known. Also the diagnosis of pulmonary aspergillosis is missed many times by the routinely done laboratory investigations. Amongst the different Aspergillus species, Aspergillus fumigatus is the predominant species, followed by Aspergillus flaus, Aspergillus niger etc. Similar results were reported by Seyedjavadi et al., (2022) and Seyed et al., (2022).

Isolation of uncontaminated bronchial secretions for culture of pathogens (Bacteria and Fungi) was done by flexible fiberoptic Bronchoscopy. Fiberoptic Bronchoscope with protected catheter brush with distal polyethylene glycol plug as devised by Hanneke et al., (2018) is the method of choice to obtain uncontaminated bronchial secretions. This was confirmed by various workers Jolin et al., (2019) and Aridgides et al., (2021). This give a perspective study of lower respiratory tract flora in patients of chronic lung diseases.

Age Distribution

The mean age of patients in our study was 52.41 ± 10.313 years and maximum numbers of patients in our study were in the age group of 51-60 years (40%). Patients above the age of 41 years constituted the bulk (80%). The range was 30-70 years. Seyedjavadi et al., (2022) analyzed 181 fungal patients with COVID-19 from 23 countries, and co-infection in the age group of over 50 years was higher than less than 50 years (66.2 vs. 23.7%) which is in agreement with studies that exhibited elderly patients have a higher risk of COVID-19 infection and mortality. Present study concordance with a study conducted in the United Kingdom on co-infection patients with COVID-19 symptoms, which reported that the highest prevalence of co-infection patients was in the age group of 55– 81 years (Seyedjavadi et al., 2022). Senok et al., (2021) found that the mean age of patients with co-infections was 49.3 ± 12.5 years in the United Arab Emirates. These observations indicated that declined immune system ability and increasing comorbid conditions with age could be a rational justification for the observed increased infection in older patients (Seyedjavadi et al., 2022).

The mean age in these studies varied as 53.4, and 49.1 years respectively. In a study reported by Seyed et al., (2022) the mean age at presentation was 73.8 years (range: 45–88 years; median=77; IQR=18).

Sex Distribution

Out of the fifty patients, majority of them were Males (68%) with a Male: Female ratio of 2.12:1 COPD is described as a disease of Males. Seyedjavadi et al., 2022 reported that the patients' gender was assessed in 71 studies that indicated COVID-19 infection in men (72.9%) was higher than that of women (25.9%) (Seyedjavadi et al., 2022).

While Seyed et al., (2022) reported (70.6%) were males and 5 (29.4%) were females. In favor of males, the incidence of the disease in Females is increasing due to increasing trend of smoking in females.

Age at Onset of Disease

The mean age of onset of the disease in study patients was 46.9 ± 8.43 years. Majority of patients (90%) had the disease onset between the ages of 31-60 years. Evangelia et al., (2022) have also reported similar findings in their study with maximum number of patients having their age of onset of disease between 43-70 years (Evangelia et al., 2022).

Duration of Illness:

Cough and expectoration was present in these patients for a mean duration of 5.35 ± 2.67 years 50% patients had a total duration less than 7 years while another 30% patients had it for 7-10 years. 2 (4%) patients had dyspnoea for duration of 3-7 years.

Isolated Fungal:

Different fungal species were cultured in 26% total cases. 20% isolates were positive for fungi alone, whereas in 6% cases, both bacteria and Fungi were present. Among fungi Candida was the predominant isolate in 16% cases followed by Aspergillus species (8%) and Histoplasma capsulatum in just one (2%) cases. Fungi were isolated in bronchial aspirates of bronchiectatic patients.

There is high incidence of Mycotic involvement in patients of chronic lung diseases specially pulmonary tuberculosis, Bronchiectasis while incidence of mycoses is less in patients of other chronic lung diseases like chronic obstructive lung disease, bronchial asthma etc unless the patient is Immunocompromised due to prolonged antibiotic administration, or corticosteroid treatment, diabetes mellitus etc. the pulmonary mycoses are accrued by inhalation of Mycelial elements and spores of pathogenic fungi normally found as saprobes growing in soil decaying matter or other natural substances (Russo et al., 2020; Spinello et al., 2018). Advances in modern treatment like Antibiotics corticosteroids immunosuppressants and anticancer drugs have led to increase opportunistic fungal infection including bronchopulmonary (Azar et al., 2017; Martin et al., 2022; Li JX et al., 2017). In India there is high prevalence of chronic lung diseases: still the diagnosis of aspergillosis and Candidiasis is usually not made: due to lack of mycological tests in routine diagnostic laboratories.

Conclusion:-

From the above observations, following conclusions were drawn.

In COVID-19 PATIENTS which are already containing Chronic lung diseases especially chronic obstructive pulmonary disease, bronchiectasis is more common in elderly males. The Bronchiectatic patients are also at risk of getting, fungal infections (Aspergillus species / Candida species etc.) Patients of chronic lung disease who are on long-term antibiotic therapy, and or steroids are more predisposed to get these opportunistic infections.

From these conclusions it is clear that there is great need to undertake more exhaustive studies on course of the disease, pathogenesis, role of genetic factors, influence of environmental factors, guidelines for health care providers regarding effective ways of managing disease, research to gauge the impact and reduce the risk from growing air pollution, urbanisation, recurrent childhood infections and occupational exposures.

Large number of patients should be studied in such study, more research on the role of viral infections in infancy and childhood, role of anaerobic infections in the chronic lung diseases is needed increasing use of fiberoptic bronchoscopy both in normal persons and quiescent phase of the chronic lung diseases to know the significance of bacterial isolation, common patterns of colonisation by respiratory pathogens, better and judicious use of antibiotics in patients to prevent drug resistance, to prevent further progression of the diseases.

Author Contribution:

Dr. Sanjay Kumar Sharma^{5*} was the main researcher who chose topics, searched, and collected study data. Mr. Dharendra Kumar Singh¹, Dr. Rajesh Sharma², Dr. Brajesh ranjan & Dr. Rajiv Srivastava⁴, analyzed and reviewed the study documents.

Funding and Sponsorship:

This research used personal funds.

Acknowledgement:-

The researcher would like to send his gratitude to the publishers and to the RDMC Principal Sir, for database providers.

Conflict Of Interest:

There was no conflict of interest in the study.

References:-

1. Lum, L., Lee, A., Vu, M., Strasser, S., & Davis, R. (2020). Epidemiology and risk factors for invasive fungal disease in liver transplant recipients in a tertiary transplant center. *Transplant Infectious Disease: An Official Journal of the Transplantation Society*, 22(6), e13361. <https://doi.org/10.1111/tid.13361>.
2. Singla, N., Bhanhur, D., Aggarwal, D., & Chander, J. (2019). Prevalence of allergic bronchopulmonary aspergillosis among patients with severe bronchial asthma in a tertiary care hospital in Northern India. *Indian Journal of Pathology & Microbiology*, 62(1), 111. https://doi.org/10.4103/ijpm.ijpm_205_18
3. Russo, A., Tiseo, G., Falcone, M., & Menichetti, F. (2020). Pulmonary aspergillosis: An evolving challenge for diagnosis and treatment. *Infectious Diseases and Therapy*, 9(3), 511–524. <https://doi.org/10.1007/s40121-020-00315-4>.
4. Antinori, S., Corbellino, M., & Parravicini, C. (2018). Challenges in the diagnosis of invasive fungal infections in immunocompromised hosts. *Current Fungal Infection Reports*, 12(1), 12–22. <https://doi.org/10.1007/s12281-018-0306-0>.
5. Azar, M. M., & Hage, C. A. (2017). Clinical perspectives in the diagnosis and management of histoplasmosis. *Clinics in Chest Medicine*, 38(3), 403–415. <https://doi.org/10.1016/j.ccm.2017.04.004>.
6. Scurek, M., Pokojova, E., Doubkova, M., & Brat, K. (2022). Allergic bronchopulmonary candidiasis: A review of the literature and a case report. *BMC Pulmonary Medicine*, 22(1), 132. <https://doi.org/10.1186/s12890-022-01921-3>.
7. Li, J. X., Fan, L. C., & Li, M. H. (2017). Beneficial effects of Omalizumab therapy in allergic bronchopulmonary aspergillosis: a synthesis review of published literature. *Resp Med*, 122, 33–42.
8. Sanchez, P., Velez-Del-Burgo, A., Sunen, E., Martínez, J., & Postigo, I. (2009). Fungal Allergen and Mold Allergy Diagnosis: Role and Relevance of *Alternaria alternata* Alt a 1 Protein Family. *J Fungi (Basel)*. 8.
9. Mansour, O., & Rabab, A. E. (2019). Spectrum of fungal infection in pulmonary intensive care unit of Menoufia University Hospital Medicine. *Biology*.
10. Zuo, Y.-H., Wang, W.-Q., Chen, Q.-J., Liu, B., Zhang, F.-Y., Jin, X.-Y., Hang, J.-Q., Li, H.-Y., Bao, Z.-Y., Jie, Z.-J., Wang, G.-F., Gao, X.-W., Sun, H., Xu, J.-F., Zhang, J., & Qu, J.-M. (2020). *Candida* in lower respiratory tract increases the frequency of acute exacerbation of chronic obstructive pulmonary disease: A retrospective case-control study. *Frontiers in Cellular and Infection Microbiology*, 10, 538005. <https://doi.org/10.3389/fcimb.2020.538005>.
11. Hamdy, M., & Wageeh, H. (2020). Effect of Corticosteroid Therapy in Patients With an Acute Exacerbation of Chronic Obstructive Pulmonary Disease Receiving Ventilatory Support *MJMR*. 31, 133–141.
12. Gross-Rother, J., Blech, M., Preis, E., Bakowsky, U., & Garidel, P. (2020). Particle detection and characterization for biopharmaceutical applications: Current principles of established and alternative techniques. *Pharmaceutics*, 12(11), 1112. <https://doi.org/10.3390/pharmaceutics12111112>.
13. Karahalil, E., Coban, H. B., & Turhan, I. (2019). A current approach to the control of filamentous fungal growth in media: microparticle enhanced cultivation technique. *Critical Reviews in Biotechnology*, 39(2), 192–201. <https://doi.org/10.1080/07388551.2018.1531821>.
14. Mackie, M. C., Cartney, J., & Mackie, M. C. (1996). *Practical Medical Microbiology*. Practical Medical Microbiology.
15. Sadat Seyedjavadi, S., Bagheri, P., & Nasiri, M. J. (2022). Fungal Infection in Co-infected Patients With COVID-19: An Overview of Case Reports/Case Series and Systematic Review. 13.
16. Erami, M., Hashemi, S. J., Raiesi, O., Fattahi, M., Getso, M. I., Momen-Heravi, M., Daie Ghazvini, R., Khodavaissy, S., Parviz, S., Mehri, N., & Babaei, M. (2022). COVID-19-associated pulmonary aspergillosis (CAPA) in Iranian patients admitted with severe COVID-19 pneumonia. *Infection*. <https://doi.org/10.1007/s15010-022-01907-7>.

17. Eyns, H., Piérard, D., De Wachter, E., Eeckhout, L., Vaes, P., & Malfroot, A. (2018). Respiratory bacterial culture sampling in expectorating and non-expectorating patients with cystic fibrosis. *Frontiers in Pediatrics*, 6, 403. <https://doi.org/10.3389/fped.2018.00403>.
18. Wong, J., Lee, J. S. E., Wong, T. G. L., Iqbal, R., & Wong, P. (2019). Fiberoptic intubation in airway management: a review article. *Singapore Medical Journal*, 60(3), 110–118. <https://doi.org/10.11622/smedj.2018081>.
19. Aridgides, D., Dessaint, J., Atkins, G., Carroll, J., & Ashare, A. (2021). Safety of research bronchoscopy with BAL in stable adult patients with cystic fibrosis. *PloS One*, 16(1), e0245696. <https://doi.org/10.1371/journal.pone.0245696>.
20. Hughes, S., Troise, O., Donaldson, H., Mughal, N., & Moore, L. S. P. (2020). Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, 26(10), 1395–1399. <https://doi.org/10.1016/j.cmi.2020.06.025>.
21. Senok, A., Alfaresi, M., Khansaheb, H., Nassar, R., Hachim, M., Al Suwaidi, H., Almansoori, M., Alqaydi, F., Afaneh, Z., Mohamed, A., Qureshi, S., Ali, A., Alkhajeh, A., & Alsheikh-Ali, A. (2021). Coinfections in patients hospitalized with COVID-19: A descriptive study from the United Arab Emirates. *Infection and Drug Resistance*, 14, 2289–2296. <https://doi.org/10.2147/idr.s314029>.
22. Koukaki, E., Rovina, N., Tzannis, K., Sotiropoulou, Z., Loverdos, K., Koutsoukou, A., & Dimopoulos, G. (2022). Fungal infections in the ICU during the COVID-19 era: Descriptive and comparative analysis of 178 patients. *Journal of Fungi (Basel, Switzerland)*, 8(8), 881. <https://doi.org/10.3390/jof8080881>.