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Gene Reports 27 (2022) 101588

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Fungal and bacterial co-infections of the respiratory tract among patients with COVID-19 hospitalized in intensive care units

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ARTICLE INFO

Edited by Christine Thien

Keywords:
Bacterial infections
Fungal infection
Respiratory tract
COVID-19
Iran
Coinfection

ABSTRACT

Backgrounds: The pandemic of COVID-19 has created a global public health crisis. ICU patients with COVID-19 are prone to infections of bacterial and/or fungal origins due to several risk factors. Consequently, the current study was conducted to evaluate the frequency, demographic characteristics, underlying conditions, and etiologic agents of fungal and bacterial co-infections of the respiratory tract among ICU patients with COVID-19 in Iran.

Materials and methods: From May to October 2020, sputa and endotracheal aspirates were collected from ICU patients hospitalized with COVID-19 who also were suspected of bacterial and/or fungal co-infections according to inclusion criteria. The etiologic agents of bacterial co-infections were identified using the Vitek 2 identification method. For fungal identification, all samples were analyzed by direct microscopy using KOH 10% and culture. Furthermore, all isolates were subjected to sequencing method.

Results: A total of 73 lung specimens were obtained from patients who met the inclusion criteria. Of these, in 15 cases (20.54%) fungal and/or bacterial co-infections were confirmed. Males were more infected (73.33%) and all of them were between 49 and 79 years. *Candida albicans* (n = 8, 61.53%) and *Klebsiella pneumoniae* (n = 5, 38.46%) were the most frequent etiologic agents related to fungal and bacterial co-infections, respectively. Pneumonia (n = 15, 100%) and diabetes mellitus (n = 8, 53.33%) were documented as the most prevalent underlying conditions. In the current study, 3 out of 15 patients (20%) died.

Conclusion: The frequency of bacterial co-infections of the respiratory tract in ICU patients hospitalized with COVID-19 was relatively high. According to the results, one of the causes of death of these patients could be a secondary infection.

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, Severe Acute Respiratory Coronavirus 2; SDA, Sabouraud Dextrose Agar; BHI, Brain Heart Infusion.

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https://doi.org/10.1016/j.genrep.2022.101588
Received 8 October 2021; Received in revised form 3 March 2022; Accepted 3 March 2022
Available online 8 March 2022

1. Introduction

The world is currently witnessing a pandemic of coronavirus disease 2019 (COVID-19), which is described as the most detrimental infectious-disease crisis since the influenza pandemic in 1918 (Clancy and Nguyen, 2020). Until December 22, 2020, there have been a total of 77,471,325 global confirmed cases of COVID-19 with 1,705,008 global deaths related to this infection (Center, n.d.). The pathogen responsible for the infection, *Severe Acute Respiratory Coronavirus 2* (SARS-CoV-2), is an enveloped RNA beta coronavirus (Lu et al., 2020) which is phylogenetically related to SARS-CoV-1 (Lansbury et al., 2020). During the 1918 and subsequent influenza pandemics, co-infections were common causes of mortality and morbidity, in fact, it was reported that as much as 95% of the mortality of the "1918 Spanish flu" is attributed to bacterial co-infections (Cauley and Vella, 2015). Furthermore, a fungal or bacterial co-infection was identified in almost a quarter of H1N1 patients in the 2009 pandemic (MacIntyre et al., 2018 Dec). Additionally, aspergillosis along with other fungal infections are underreported complications of severe influenza (Salehi et al., 2021; Schauwvlieghe et al., 2018; Van De Veerdonk et al., 2017; Rijnders et al., 2020; Koehler et al., 2020).

Bacterial and/or fungal secondary infections are important factors affecting the prognosis and mortality of COVID-19 patients (Ruan et al., 2020 May). The most commonly encountered pathogen in COVID-19 patients with co-infections is *Streptococcus pneumoniae*, followed by *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Aspergillus* (Zhu et al., 2020). However, there are insufficient data about infections secondary to COVID-19 (Zhou et al., 2020a). Most of the current infection control protocols aim to prevent the transmission of the COVID-19 virus while underrating the importance of prevention of secondary bacterial or fungal infections. When, in reality, secondary infections were found in half of non-survivor COVID-19 patients (Zhou et al., 2020b). Nonetheless, even in the limited available studies with data about secondary infections, a discrepancy between empiric antimicrobial prescribing (72%–100%) and the reported incidence of secondary infection (8%–15%) has been detected (Zhou et al., 2020b; Rawson et al., 2020; Huang et al., 2020). Given the aforementioned mismatch, recent World Health Organization guidelines recommend empiric antibiotics only for patients with severe COVID-19, using host-factors and local epidemiology to drive antibiotic selection (World Health Organization, 2020).

Clinical data about bacterial and fungal co-infections is valuable to guide the evidence-based treatment of COVID-19. Consequently, we undertook a cross-sectional analysis of ICU patients admitted with confirmed SARS-CoV-2 at our medical center, where we sought to characterize the frequency, demographic characteristics, underlying conditions, and etiologic agents of bacterial and fungal co-infections of the respiratory tract in Iranian ICU patients hospitalized with COVID-19 during the pandemic.

2. Material and methods

2.1. Ethics statement

Informed consent to take part in the study has been obtained from all participants. Ethics committee approval was received for this study from the ethics committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1399.267).

2.2. Study population, clinical data, sample collection and processing

This was a cross-sectional single-center study involving ICU hospitalized patients at *Imam* Khomeini Hospital Complex, a referral tertiary center in Tehran, Iran. During a period of 6 months (from May to October 2020), lung specimens were obtained from ICU hospitalized patients with a positive real-time PCR test for COVID-19 who also were suspected of bacterial and/or fungal co-infections. One respiratory sample was obtained from each patient. The patients have had at least two of the hereunder conditions:

(1) patients receiving immunosuppressants,

(2) having clinical symptoms of pulmonary fungal and/or bacterial infections reported by a specialist in fields of pulmonary diseases (dyspnea, cough, fever, chest pain, purulent sputum, weight loss, hemoptysis, and wheezing),

(3) suspicious radiographic findings indicating a pulmonary fungal and/or bacterial infection according to a pulmonologist opinion.

With the intention of preventing false-negative results, patients with a history of receiving systemic antifungal and/or antibiotic therapy or prophylaxis within the last one month before admission were excluded from the study. Also, patients from whom no respiratory sample could be obtained were excluded.

Once collected, the specimens were rapidly transported to the laboratory, and microscopic and culture experiments performed on the specimens for less than one hour. Sputum samples were diluted by adding sterile distilled water (10 μL of sputum in 5 mL of sterile distilled water) and vortex mixing. Also, endotracheal aspirates were centrifuged (at 1200 $\times g$ for 10 min) and all but 0.5 mL of supernatant was tipped-off. Then, centrifuged deposit in the remaining fluid was re-suspended.

Demographic data (gender, age), underlying health conditions, and outcomes including hospital discharge and in-hospital death were recorded.

According to the host factors (clinical symptoms, radiology findings and predisposing factors which were defined as inclusion criteria), and the positive results in direct examination (for fungal infections) and culture (for both fungal and bacterial infections) the infection was confirmed.

2.3. Bacterial and fungal identification

For bacterial identification the specimens were inoculated to eosin methylene blue (EMB) agar and chocolate agar media and incubated overnight at 37° C. For sputa, 1 μL loopful of the final dilution prepared in 2.2 was inoculated to each type of media plate, and for endotracheal aspirates using a sterile loop, each media plate was inoculated with the deposit of the centrifuged sample (England, n.d.). The isolates were then Gram stained and identified at the species level using the Vitek 2 (bioMerieux, Marcy l'Etoile, France) method according to the manufacturer's instructions. For fungal agents, KOH 10% solution was used to dissolve the samples to observe under a microscope. All specimens were cultured on Sabouraud Dextrose Agar (SDA) with chloramphenicol and Brain Heart Infusion (BHI) agar media (Merck, Germany). Dilution of samples used for inoculation to fungal media plates was similar to the dilutions used for inoculation on bacterial media plates (England, n.d.). Furthermore, for confirmation of fungal identification, all isolates were subjected to sequencing method. Briefly, DNA was extracted using High Pure PCR Template Preparation kit (Roche, Germany) according to the recommended instructions of the manufacturer. A fragment of the ITS gene was amplified using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) primers in the following thermal conditions: 95 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 45 °C for 30 s, and 72 °C for 45 s, followed by one final extension at 72 °C for 5 min (Kamali Sarwestani et al., 2019). PCR products were subjected for single direction sequencing using forward primer (Bioneer, South Korea). The results were checked visually using Chromas version 3.5.1 (http://technelysium.com.au/wp) and were deposited to the GenBank. The species of each isolate was identified in comparison to GenBank reliable sequences using basic local alignment search tool of the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All of the sequences had been deposited in GenBank under the accession number reported in Table 1.

In this study, criteria such as presence of invasive forms (numerous budding yeasts, pseudohyphae and true hyphae) in direct examination or significant growth of pure creamy mucoid colonies on culture media were considered to distinguish between *Candida* colonization and

Table 1
GenBank accession numbers of DNA sequences included in this study.

Isolate	Molecular identification (IT'S[a] gene)	GenBank accession number
SP1[b]	*Candida glabrata*	MT772038
EA1[c]	*C. glabrata*	MT772039
EA2	*Candida.albicans*	MT772040
EA3	*Candida krusei*	MT772041
EA4	*C. albicans*	MT772042
EA5	*C. krusei*	MK793223
EA6	*C. albicans*	MT772043
SP2	*C. albicans*	MT772044
EA7	*C. albicans*	MT772045
EA8	*C. albicans*	MT772046
SP3	*C. glabrata*	MK793225
EA9	*C. albicans*	MT772047
EA10	*C. albicans*	MT772048

ITS [a]: internal transcribed spacer; SP [b]: Sputum; EA [c]: Endotracheal aspirate.

infection of the respiratory tract in patients who met the inclusion criteria. Diagnostic thresholds for lung specimen analysis in bacterial and fungal infections of the respiratory tract were 10^5–10^6 CFU/mL for sputa and 10^4 CFU/mL for endotracheal aspirates (England, n.d.).

2.4. Statistical analysis

Statistical analysis was performed with SPSS-v.24 (IBM, Chicago, IL). We summarized the collected data by using descriptive statistics, presenting continuous variables with normal distribution as mean (standard deviation [SD]); non-normal variables as median (interquartile range [IQR]), and categorical variables as proportions or percentages.

3. Results

During the period of the current study (May 2020 until October 2020), totally 73 ICU hospitalized patients with positive real-time PCR tests for COVID-19 were suspected of fungal and/or bacterial co-infections according to inclusion criteria. A total of 73 lung specimens including 33 sputa and 40 endotracheal aspirates were collected. From 73 patients included in this study, the majority were male (n = 48, 65.75%) with the median age of 52 years (range 15–89 years). Fungal and/or bacterial co-infections were confirmed in 15 cases (20.54%). Regarding the type of infection, 73.34% (n = 11) of the patients had mix infections (simultaneous bacterial and fungal infections), also 13.33% of the patients (n = 2) only had bacterial co-infection, and 13.33% of the patients (n = 2) only had fungal co-infection (Table 2). The 15 patients in the current study comprised 11 men (73.33%) and 4 women (26.66%) with the median age of 64 years (range 49–79 years). All of the patients (n = 15, 100%) had pneumonia as an underlying condition. Diabetes mellitus was documented as the second most prevalent underlying condition as 8 (53.33%) patients were diabetic. Among the etiologic agents responsible for bacterial co-infections *Klebsiella pneumoniae* (n = 5, 38.46%) was the most frequent species detected from the respiratory tract of ICU patients hospitalized with COVID-19 who also were

Table 2
Detailed information of each ICU patient hospitalized with COVID-19 who had positive results for fungal and/or bacterial co-infections of the respiratory tract.

Patient number	Age (years)	Gender	Underlying diseases	Sample	CT scan[b]	Fungal culture and molecular detection	Bacteria	Antifungal and/or antibacterial given for curative intent	Outcome
1	51	Male	Pneumonia, heart failure and hypertension, lung cancer	Sputum	–	*Candida glabrata*	*Streptococcus viridans*	caspofungin+ Meropenem	Recovered
2	51	Male	Pneumonia, diabetes mellitus, AIDS[a]	Endotracheal aspirate	Nodular infiltrates	*Candida glabrata*	*Klebsiella pneumoniae*	Amphotericin B + Meropenem	Recovered
3	53	Female	Pneumonia, diabetes mellitus, systemic lupus erythematosus	Sputum	Grund-glass opacities	None	*K. pneumoniae*	Meropenem	Recovered
4	74	Male	Pneumonia, rheumatoid arthritis	Endotracheal aspirate	Nodular infiltrates	*Candida albicans*	*K. pneumoniae*	Fluconazole+ Meropenem	Recovered
5	73	Male	Pneumonia, prostate cancer	Endotracheal aspirate	–	*Candida krusei*	*Acinetobacter baumannii*	Fluconazole+ Meropenem	Recovered
6	49	Male	Pneumonia, rheumatoid arthritis	Endotracheal aspirate	–	None	*K. pneumoniae*	Meropenem	Recovered
7	79	Male	Pneumonia, diabetes mellitus	Endotracheal aspirate	–	*C. albicans*	*A. baumannii*	Fluconazole+ Meropenem	Died
8	67	Male	Pneumonia, rheumatoid arthritis	Endotracheal aspirate	Nodular infiltrates	*C. krusei*	None	Fluconazole	Recovered
9	60	Female	Pneumonia, multiple sclerosis	Endotracheal aspirate	–	*C. albicans*	S. viridans	Fluconazole+ Meropenem	Recovered
10	74	Male	Pneumonia, diabetes mellitus, prostate cancer	Sputum	–	*C. albicans*	*Proteus vulgaris*	Fluconazole+ Meropenem	Recovered
11	65	Female	Pneumonia, Lung cancer	Endotracheal aspirate	–	*C. albicans*	None	Fluconazole	Recovered
12	57	Female	Pneumonia, diabetes mellitus, breast cancer	Endotracheal aspirate	Nodular infiltrates	*C. albicans*	*A. baumannii*	Fluconazole+ Meropenem	Died
13	55	Male	Pneumonia, diabetes mellitus, rheumatoid arthritis	Sputum	–	*Candida glabrata*	*Klebsiella oxytoca*	Fluconazole+ Meropenem	Recovered
14	46	Male	Pneumonia, diabetes mellitus, prostate cancer	Endotracheal aspirate	Bilateral ground-glass opacities	*C. albicans*	*A. baumannii*	Fluconazole+ Meropenem	Recovered
15	60	Male	Pneumonia, diabetes mellitus, prostate cancer	Endotracheal aspirate	–	*C. albicans*	*K. pneumoniae*	Fluconazole+ Meropenem	Recovered

[a] AIDS, Acquired immunodeficiency syndrome.
[b] CT scan, computerized tomography.

suspected of fungal and/or bacterial co-infections, followed by *Acinetobacter baumannii* (n = 4, 30.77%), *Streptococcus viridans* (n = 2, 15.39%), *Klebsiella oxytoca* (n = 1, 7.69%), and *Proteus vulgaris* (n = 1, 7.69%).

Also, among the etiologic agents responsible for fungal co-infections, *Candida albicans* (n = 8, 61.53%) was the most frequent detected species, followed by *Candida glabrata* (n = 3, 23.08%), and *Candida krusei* (n = 2, 15.39%).

In the current study, 3 out of 15 patients (20%) died and 12 patients (80%) recovered.

Detailed information of each ICU patient hospitalized with COVID-19 who had positive results for fungal and/or bacterial co-infections of the respiratory tract are provided in Table 2.

Also, in the current study, all results related to the molecular characterization of isolated fungal elements to the species level were in accordance with the results of mycological methods.

4. Discussion

The respiratory tract is the most prevalent site for developing fungal or bacterial infections. In majority of patients, colonization is the first step in pathogenesis of pulmonary infections with fungal or bacterial pathogens (Kamali Sarvestani et al., 2021; Rafat et al., 2020a; Rafat et al., 2020b). Furthermore, due to several risk factors, patients with COVID-19 hospitalized in intensive care units are susceptible to infections caused by bacterial and/or fungal elements. In this study, we used 73 lung specimens related to ICU patients hospitalized with positive real-time PCR tests for COVID-19 who also were suspected of bacterial and/or fungal co-infections according to a pulmonologist opinion to make a preliminary assessment of the clinical characteristics of patients with COVID-19 from the following aspects such as age, gender, underlying disease and the type of respiratory co-infection. We found 15 COVID-19 cases were co-infected with other respiratory pathogens, especially *Candida albicans* and *Klebsiella pneumonia*. *Klebsiella pneumoniae* has emerged as a significant opportunistic pathogen responsible for nosocomial infections. This bacterium is frequently resistant to multiple classes of antibiotics such as family of beta-lactam antibiotics. On the other hand, the polymorphic fungus *Candida albicans* is a member of the normal flora of the respiratory tract which in some conditions can be the most prevalent cause of candidiasis. The global incidence of candidiasis has increased in the last decade as a result of the change in the frequency of at-risk population, increase in the administration of broad-spectrum antibacterial agents, increase in the use of invasive methods (i.e., parenteral nutrition, peripheral vascular angioplasty, atherectomy, stents, mechanical ventilation, cardiac catheterization), use of complex surgical procedures, and immunosuppressive therapy. These conditions are common among patients with infections caused by COVID-19. The ability to grow in both yeast and hyphal forms, the production of secreted proteinase activity, and the ability to biofilm formation are important virulence factors related to this microorganism (Sasani et al., 2021).

In a report of 99 patients with COVID-19 in China, Chen et al. (2020a) documented 2 patients with significant growth in their sputa. One patient had a polymicrobial infection with *A. baumannii, Klebsiella pneumoniae*, and *Aspergillus fumigatus* isolated from either sputum or tracheal aspirate. From the sputum sample of the second patient *Candida albicans* was isolated. Wang et al. (2020) reported 69 patients with COVID-19 undergoing sputum culture on admission to the hospital to identify respiratory bacterial or fungal co-infections. Of these, 5 of 69 (7.25%) had positive culture results, including *Candida albicans* (2/5, 40%), *Enterobacter cloacae* (2/5, 50%), and *A. baumannii* (1/5, 20%). Also, Zhou et al. (2020c) reported observation of secondary bacterial infection in 28 of 191 (14.66%) patients admitted to hospitals in China. Of these patients with secondary bacterial infection, 27 of 28 died. No further details on the type of infection, methods of identification, and healthcare setting were provided. Hughes et al. (2020) found a low frequency of bacterial co-infection in patients hospitalized with COVID-19. Also, they found no evidence of respiratory fungal infection until 5 days after admission in these patients.

Diabetes mellitus was found to be an important underlying condition for fungal and/or bacterial co-infection in this study. Similarly, in previous studies, diabetes was the main reported chronic underlying diseases in patients with COVID-19, being more prevalent among patients with severe disease and also associated with poor prognosis (Guan et al., 2020; Yang et al., 2020a). Diabetes patients have an immune system with a lower ability to respond to and deal with diseases of any type. This means they are more prone to illnesses than the general population. Studies showed that diabetes is correlated to the development of fungal/bacterial/viral pneumonia, tuberculosis and chronic obstructive pulmonary disease (COPD) (Rafat et al., 2020b; Ramirez et al., 1991).

Our results showed that the median age of the 15 patients in the current study was 64 years which is in accordance with the results of other studies (Ling et al., 2020; Bhatraju et al., 2020; Yang et al., 2020b). The combination of predisposing factors and the decrease in the activity of the immune system can make these age groups more prone to bacterial or fungal infections.

During our experiences in the management of patients with COVID-19, in accordance with other studied (Ling et al., 2020; Bhatraju et al., 2020; Yang et al., 2020b; Chen et al., 2020b) we found that males were more affected than females by bacterial and/or fungal co-infections of the respiratory tract.

We demonstrated a relatively high frequency of respiratory bacterial and fungal co-infections in critically ill COVID-19 patients admitted to the ICU. Careful examinations and necessary tests should be performed to exclude co-infections in order to appropriately treat critically ill ICU COVID-19 patients.

In conclusion, the present study emphasizes the concern of bacterial and fungal infections among ICU patients hospitalized with COVID-19. *Klebsiella pneumonia* (bacterium) and *Candida albicans* (fungus) were the most frequent pathogens detected from the respiratory tract. Diabetes mellitus, gender, and age group were found to be important underlying conditions for microbial coinfection. Further large-sample, well-designed studies are warranted to investigate the prevalence of COVID-19 co-infection, risk of co-infection, microbiological distribution, and impact of co-infection on the clinical outcomes of COVID-19 patients. It should be noted major limitation of the present study is the fact that we did not include a control group (COVID-19 negative participants) that can be considered in future studies.

CRediT authorship contribution statement

Zahra Rafat: Writing – original draft, Conceptualization, Methodology, Resources, Data curation. **Alireza Ramandi:** Investigation. **Pegah Afarinesh Khaki:** Investigation. **Saham Ansari:** Writing – original draft, Investigation. **Sara Ghaderkhani:** Investigation. **Hassan Haidar:** Investigation, Visualization. **Faezeh Tajari:** Software, Formal analysis. **Davoud Roostaei:** Writing – review & editing. **Roshanak Daei Ghazvini:** Resources, Visualization, Data curation. **Seyed Jamal Hashemi:** Methodology, Investigation. **Alireza Abdollahi:** Project administration, Funding acquisition. **Hasti Kamali Sarvestani:** Conceptualization, Validation, Supervision.

Declaration of competing interest

The authors have no conflicts of interest to declare for this study.

Acknowledgments

The authors wish to thank the financial support of Tehran University of Medical Sciences, Tehran, Iran.

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