Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

desirable that actions aimed at reducing mortality in patients with COVID-19, should take into account the worsening of the patient's clinical condition due to secondary infections caused by multi-resistant microorganisms. Therefore, epidemiological studies with antimicrobial surveillance systems that promote the production of quality evidence about antimicrobial intervention effectiveness in patients with COVID-19, especially in critically ill patients in intensive care units should be encouraged.

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Bacterial and fungal colonization of the respiratory tract in COVID-19 patients should not be neglected

To the editor:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily affects the respiratory system, although the involvement of other organs, such as heart, kidney, and bowel, has also been observed.[1-5] We conducted a retrospective study on 61 patients with coronavirus disease 19 (COVID-19) admitted to Desio Hospital, Lombardy, Italy, from February 1, 2020 to April 30, 2020 to assess bacterial and fungal pulmonary colonization. In accordance with WHO guidance, only SARS-CoV-2 cases confirmed through real-time reverse-transcriptase–polymerase-chain-reaction assays on nasopharyngeal swabs were included in the analysis.[6] All patients were admitted to the intensive care unit (ICU) due to the presence of acute hypoxemic respiratory failure that required invasive respiratory supports, such as mechanical ventilation and high level of positive end-expiratory pressure. Here, we investigated the relationship between SARS-CoV-2 and bacterial and fungal colonization using data of first bronchial aspirate cultures of each patient, 35 (57%) of which resulted positive for bacterial or fungal infection. The species identification was performed by Vitek Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry system (bioMérieux). Pathogenic fungi were isolated in 19 specimens: 14 *Candida albicans* (40%), 4 *Candida glabrata* (11.4%), and 1 *Aspergillus fumigatus* (3%). In other 13 samples, we identified *Pseudomonas aeruginosa* (n = 6, 17%), *Staphylococcus aureus* (n = 2, 5%), *Klebsiella pneumoniae* (*n* = 1, 3%), *Escherichia coli* (*n* = 1, 3%), *Klebsiella oxytoca* (*n* = 1, 3%), *Enterobacter cloacae* (*n* = 1, 3%), or *Staphylococcus epidermidis* (n = 1, 3%). Finally, in 3 (8.6%) samples, we identified both *C. albicans* and *P. aeruginosa*. Antimicrobial susceptibility and resistance detection of the clinical isolates were determined using Vitek cards (bioMérieux, Marcy l'Etoile, France). No multidrug- resistant bacteria or fungi were isolated. Reviewing the antimicrobial resistance data of bronchial aspirate isolates of patients hospitalized in the ICU in 2019, we found that 17% of the isolated strains was resistant to beta-lactam drugs, vancomycin, carbapenem drugs, or methicillin. This observation is in contrast with the results observed for the strains isolated from severe COVID-19 patients, suggesting that the bacterial and fungal colonization in these subjects was not of nosocomial origin.

In this study, among the 35 patients presenting lung SARS-CoV-2 infection and concomitant positive bronchial aspirates, 28 (80%) were colonized by either fungi or *P. aeruginosa.* Fungi are the major causes of morbidity and mortality in immunocompromised subjects, while *P. aeruginosa* is the most common gram-negative pathogen causing pneumonia associated with worse clinical outcomes.[7,8] On the other hand, in non-COVID-19 patients admitted to the ICU in 2019, only 20% of first bronchial aspirate cultures presented fungi (*C. albicans, C. glabrata, C. tropicalis, C. parapsilosis*, and *A. fumigatus*) or *P. aeruginosa* colonization.

SARS-CoV-2 enters the target cells via the angiotensin-converting enzyme 2 receptor, which is highly expressed on alveolar epithelial cells, but also on heart, kidney, and intestinal cells.[3,9] A constellation of innate immune cells (neutrophils and monocytes) and adaptive immune cells (particularly $CD4^+$ T cells and $CD8^+$ T cells) are involved in the response to viral infections, and COVID-19 is no exception. It has been reported that SARS-CoV-2 strongly activates the immune system inducing an abnormal cytokine production known as "cytokine storm," especially in severe cases.[10] Indeed, subjects with severe COVID-19 exhibited increased plasma levels of pro-inflammatory cytokines and chemokines, and reduced T-cells number in peripheral blood, potentially as consequence of lymphocyte accumulation to the site of infection.[3,11] Such elevated immune response can kill infected cells but can also contribute to the aggravation of the disease and to lung injury.[3,11] It is worth noticing that, in severe COVID-19 patients, beside respiratory symptoms, thrombosis and pulmonary embolism have been observed.[3] More in-depth studies are needed to identify the molecular players involved in SARS-CoV-2 pathogenesis, which might reveal key targets to reduce or inhibit the cytokine storm.

In healthy individuals, *Candida* species and *P. aeruginosa* can colonize mucous membranes and skin, and both innate and adaptive immune cells contribute to the antifungal and antibacterial defense.[7,8,12] Neutrophils, macrophages, dendritic cells, and T- and B-lymphocytes are the major cellular players. The innate immune cells are the first line of defense, and the release of inflammatory cytokines and chemokines induces the recruitment of neutrophils from the peripheral blood.

Dendritic cells are particularly important to initiate the adaptive immune response. Among adaptive immune cells, $CD4^+$ T helper cells play a key role with Th17 being the most relevant subtype. These cells act principally at the lung mucosal barrier, and produce/release interleukin-17 (IL-17), which contributes to the enhanced organization of B- and T-cells into bronchus-associated lymphoid tissue involved in mediating secondary immune responses, and the release of antifungal β-defensins. Indeed, patients with deficiency in IL-17 production or signaling appear to be more susceptible to mucosal fungal and *P. aeruginosa* infections, as previously reported.[7,8,12]

In our hospital, we observed an increased prevalence of fungal and *P. aeruginosa* colonization in severe COVID-19 patients compared to non-COVID-19 cases. It could be speculated that this association might be the result of the over-activation of the immune system causing the failure in the regulation of the defenses against pathogens other than SARS-CoV-2, and the progression to co-infections and therefore to lung injury.

Certainly, multicenter studies with a larger number of subjects are needed to verify and improve our results. However, this study highlights the importance of not to neglect fungal and bacterial lung colonization in severe COVID-19 cases that, along with the detection of molecules involved in the immune response and in the mechanisms of the host-pathogen interaction, can be useful for the development of personalized therapies and to improve patients' management in the ICU.

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