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ORIGINAL ARTICLE

Microbiological examination of lower respiratory tract specimens in COVID-19 patients

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ABSTRACT
Bacterial and fungal infections are common complications of viral pneumonia, especially in critically ill patients. They lead to an increased need for intensive care and mortality. Data about the bacterial or fungal infection in viral pneumonia led by coronavirus are limited. The pathogens causing secondary infections in SARS patients were diverse: negative bacilli were the most common, but Candida *was also common (16). Bacterial and fungal infections in COVID-19 patients have been inadequately investigated and reported thus far. Among hundreds of articles published with clinical data, only a few have reported secondary infection, mostly without detailed pathogens. Of nine studies reporting bacterial co-infection in COVID-19 cases, 62/806 (8%) cases of bacterial/fungal co-infection were reported. A few challenges exist in diagnosing secondary infection in COVID-19 patients. These conditions have led to most hospitals to decide not to carry out routine microbiological examination in COVID-19 patients, which undermines the diagnosis and treatment of secondary infection. This article aims to evaluate of microbial spectrum of tracheal aspirates obtained in COVID-19 patients, who were admitted to the intensive care unit department in TSMU The First university Clinic between 19.03 - 13.04, 2020.*
Keywords: *COVID-19, bacteria, fungi, co-infection, pneumonia.*

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INTRODUCTION

Bacterial and fungal infections are common complications of viral pneumonia, especially in critically ill patients. They lead to an increased need for intensive care and increased mortality. In influenza patients, bacterial coinfection occurs in ~0.5% of healthy young individuals and at least 2.5% of older individuals [1]. According to the cohort study report by Zhong Nanshan *et al* [2], 20 of 90 severe acute respiratory syndrome (SARS) patients had secondary lower respiratory tract infections in 2003, which accounted for 70.6% of those critical SARS patients who underwent an invasive operation. Pathogens causing secondary infections in SARS patients were diverse: negative bacilli were the most common but *Candida* was also common [2]. Invasive pulmonary aspergillosis was another common complication secondary to influenza [3]. Among the hundreds of articles published with clinical data, only a few have reported secondary infection, mostly without detailed pathogens. Zhou and colleagues reported observation of secondary bacterial infection in 28/191 (15%) of patients admitted to hospitals in China [4]. Of these patients with secondary bacterial infection, 27/28 died [7]. No further detail on the type of infection, methods of identification, and healthcare setting were provided. In a report of 99 patients all undergoing respiratory sampling on admission in China, Chen and colleagues report two patients with significant growth in their sputum. One individual had a polymicrobial infection with *Acinetobacter baumannii, Klebsiella pneumoniae,* and *Aspergillus fumigatus* isolated from either sputum or tracheal aspirate [8]. Prior healthcare exposure and underlying respiratory conditions pre-disposing this individual are not described. The second individual with significant microbiology grew a *Candida albicans*. This organism is

not normally regarded as a pathological organism when identified in culture from sputum [9]. Wang and colleagues, reported 29 of 69 patients undergoing sputum culture on admission to hospital to identify respiratory bacterial/fungal co-infection [10]. Of these, 5/69 (7%) had positive microbiology, including Candida albicans (2/5, 40%), Enterobacter cloacae (2/5, 50%), and *Acinetobacter baumannii* (1/5, 20%). Of all studies reporting bacterial/fungal coinfection in COVID-19, very few atypical organisms were identified with *Legionella pneumophila* identified in one obstetric patient admitted in China with COVID-19 [11]. Kim and colleagues report 116 individual patients undergoing respiratory pathogen sampling for atypical organisms including *Chlamydia pneumoniae* and *Mycoplasma pneumonia* [12].The authors report no atypical bacterial co-infection identified within this cohort. Despite low rates of bacterial/fungal co-infection reported in patients with COVID-19, high rates of antimicrobial prescribing are reported. Of 2010 patients reported within these studies, 1450 (72%) received antibacterial therapy. Cao and colleagues report on 102 patients from critical and non-critical care in China [13]. Of these 101 (99%) received antibacterial therapy(13). The report 87/102 (85%) receiving quinolone therapy, 34/101 (33%) cephalosporins, and 25/102 (25%) carbapenems. No bacterial/fungal co-infection was reported in this study [13] Guan and colleagues report on 1099 patients admitted to critical and non-critical care settings in China [14]. Of these 637/1099 (58%) received antibacterial and 31/1099 (3%) antifungal therapy. No microbiology was reported in this study [14]. Complications of antimicrobial therapy were not reported in any study.

During the SARS-1 outbreak in the early 2000s, Yap and colleagues reported nosocomial infection in a series of 83 patients managed within intensive care. The authors report increased rates of Meticillin Resistance Staphylococcus aureus (MRSA), *Stenotrophomonas* spp., and *Acinetobacter baumannii* in an intensive care unit that cared for 83 SARS-1 patients during a three-month period [15]. This included 30 episodes of ventilator associated pneumonia and 23 cases of MRSA transmission.

For other coronavirus infections, bacterial/fungal co-infection were observed in 43/331(13%) of cases [16,17,18]. These co-infections were for a range of Gram-positive (10/43, 23%), Gram-negative (23/43, 53%), and atypical bacteria (10/43, 23%). No data on antimicrobial susceptibility and prescribing was reported in these studies.

Discussion Rates of bacterial or fungal co-infection reported in the current medical literature for patients presenting with coronavirus infections appear to be low. Of nine studies reporting bacterial co-infection in COVID-19 cases, 62/806 (8%) cases of bacterial/fungal co-infection were reported. Use of broad-spectrum antimicrobial therapy was widely reported with 72% of COVID-19 cases receiving antibacterial therapy.

Recent Chinese publications reported at least 10% of co-infection during COVID-19 in patients hospitalized in ICU for ARDS, among them Aspergillus infections [19]. Besides, the incidence of invasive pulmonary aspergillosis (IPA) in ICU patients admitted for severe inflammation. Besides, the particular pathophysiology of COVID-19 may also account for unprecedented comorbidity with IFI. First, the high aggressive feature of the SARS-CoV-2 virus to the lung tissue and the large bilateral alveolo-interstitial lesions make the occurrence of IFI very likely, specifically those with a primary pulmonary entry and an airborne route of infection such as IPA, pneumocystosis (PjP) and mucormycosis [20]. Second, absolute number of T lymphocytes, CD4+T and CD8+T cells are markedly lower in severe COVID-19 cases than moderate cases, associated with markedly higher levels of IL-2R, IL-6, IL-10, TNF-alpha and some other inflammatory markers [21]. We believe that microbiological diagnosis should be promptly adapted to the current unprecedented situation. In accordance with the preliminary data available concerning the occurrence of IFI in COVID-19 patients, the French High Council for Public Health (HSCP - Haut Conseil de la santé publique) recommends to systematically screen for fungal pathogens in patients admitted with pneumoniae [22]. Among the 5 first well described French patients, one is co-infected with Aspergillus flavus [23].

A few challenges exist in diagnosing secondary infection in COVID-19 patients. Although it can be difficult to distinguish bacterial or fungal infection and existing viral pneumonia based on clinical and radiological appearance, microbiological examination can add great value to diagnoses, especially sputum culture. However, this approach can pose significant risks to biosample collectors and laboratory technicians processing samples from COVID-19 patients because the virus is transmitted via virus-laden aerosols in addition to respiratory droplets and direct contact [24].Thus far, no standardized personal protection equipment (PPE) has been recommended in the guidelines in China for healthcare workers who process bacterial and fungal cultures [25]. Other problems include insufficient laboratory biosafety conditions and PPE shortages. These conditions have led to most hospitals to decide not to carry out routine microbiological examination in COVID-19 patients, which undermines the diagnosis and treatment of secondary infection.

The purpose of this study was to evaluate of microbial spectrum of tracheal aspirates obtained in COVID-19 patients.

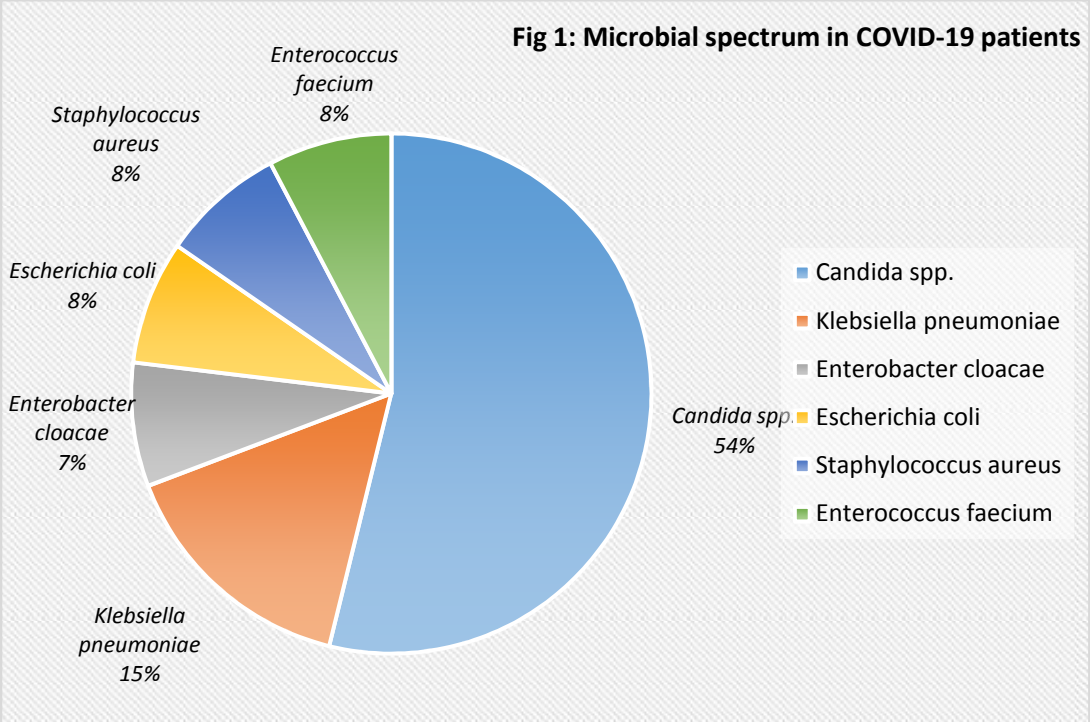
MATERIAL AND METHODS

We retrospectively studied 13 patients aged 42 to 82 years tracheal aspirates microbial spectrum, who were admitted to TSMU The First university Clinic Intensive care unit department between 19.03 - 13.04, 2020. All of them needed mechanical ventilation. Tracheal aspirates were collected and processed for culture on the day of intubation. Tracheal aspirates were examined for the identification of bacteria and fungi for their sensitivity to antibiotics. The examinations were done in Laboratory for microbiology of TSMU The First University Clinic. Samples were brought to microbiology laboratory immediately and were processed within 30 minutes of collection. If a delay of more than 1–2 h was expected, the specimen was refrigerated [26].

All specimens were cultured on blood agar, Macconkey and Sabouraud dextrose agar and incubated at 37°C for 24 h. All samples were subjected to the following: (a) gram stain of the colonies, (b) biochemical reactions by API identification system (API20E, API 20 NE, APIstaph, APIstrep, BioMérieux, France) and (C) identification and antimicrobial sensitivity test by Kirby-Bauer disk diffusion method. Gram-negative bacteria were tested for amoxicillin–clavulanic acid, piperacillin–tazobactam, ceftriaxone, ceftazidime, cefepime, meropenem, imipenem, ciprofloxacin, levofloxacin, moxifloxacin, fosfomycin and amikacin. Gram-positive bacteria were tested for amoxicillin–clavulanic acid, cefoxitin, erythromycin, linezolid, teicoplanin, and vancomycin. Fungi were tested for flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole (ATB FUNGUS3 strips manual, BioMérieux, France).

RESULTS

Results of lower respiratory tract specimens (qualified sputum, endotracheal aspirate, or bronchoalveolar lavage fluid) culture showed that the most frequent organism detected in those patients were Candida spp 54% followed by Klebsiella pneumonia 15%, Escherichia coli -8%, Staphylococcus aureus-8%, Enterococcus faecium-8%, Enterobacter cloacae-7%. Culture showed more than one organism in 15% patients (Candida spp and *Escherichia coli* [1], Enterobacter cloacae and Candida spp [1] .



DISCUSSION

Absolute number of T lymphocytes, CD4+T and CD8+T cells are markedly lower in severe COVID-19 cases than moderate cases, associated with markedly higher levels of IL-2R, IL-6, IL-10, TNF-alpha and some other inflammatory marker [21]. Airway colonization with Candida alone is associated with increased

levels of TNF-alpha and IFN-gamma within the lung, even in the histological absence of acute infection. Co-inoculation with fungi and bacteria further elevate alveolar concentrations of TNF-alpha, IFN-gamma and IL-6. As IFN-gamma is capable of impairing function of alveolar macrophages. Some have hypothesized that the presence of *C. albicans* in the airways can induce an immune response that inhibits the normal antibacterial function of host immune cells, allowing bacterial pathogens to evade clearance and initiate infection.

CONCLUSION

The present study reported high frequency airway colonization of Candida spp (54%) in COVID-19 patients. Our data is similar to the studies in China (40%) (10). However, more studies are required to determine the rate and microbial spectrum of bacterial and fungal co-infections in COVID-19 patients.

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