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Research Article

Evaluation of SARS CoV-2 RT PCR Positive Patients in Terms of Respiratory Bacterial and Fungal Infections

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Abstract

Introduction: Corona-virus disease is defined as the largest pandemic known as severe acute respiratory syndrome. Patients who are positive for SARS Cov-2 virus, the causative agent of the current pandemic outbreak, show atypical bacterial pneumonia symptoms on radiologic examinations. Secondary bacterial and fungal infections in people infected with this virus have been reported in various publications. In this study, we aimed to evaluate SARS-CoV-2 RT PCR positive patients in terms of respiratory bacterial and fungal infections. **Methods:** Between 23.03.2020 and 14.05.2023, the results of respiratory tract cultures of patients with positive SARS-CoV-2 PCR were analyzed. RT-q-19 PCR Detection Kit (Biospeedy, Turkey), SARS-CoV-2 Emerging Plus kit (Bioeksen R&D Technologies, Turkey), RTA, Ds Coronex kit were used for molecular identification. For RT-PCR, Rotorgene device (Qiagen, Germany) and CFX96 DX Real-Time PCR systems (Bio-Rad Laboratories, USA) were programmed and used according to company recommendations. **Results:** SARS Cov-2 positive patients were included in the study. Culture results were evaluated simultaneously. Of these patients with SARS COV-2 PCR test requests, 102 of them simultaneously requested culture of respiratory tract samples. Growth was detected in 61 of these. Of the patients, 38 (62%) were male and 23 (38%) were female. There were 36 (59%) patients over 65 years of age. Three (5%) *E. coli*, three (5%) *H. influenzae*, seven (11%) *P. aeruginosa*, 15 (25%) *A. baumannii*, 14 (23%) *K. pneumoniae*, three (5%) *E. cloacae*, one (2%) *S. aureus*, two (3%) *E. faecium*, two (3%) *C. striatum*, nine (15%) *C. albicans* were detected. *K. pneumoniae* and *E. faecalis* growth was detected in only two (3%) patients. **Discussion and Conclusion:** Secondary bacterial infections make viral infections more clinically complex. This is a worrying situation that negatively affects both the life expectancy and the recovery time of patients.

Keywords: SARS C0V-2; RT PCR; Secondary Bacterial Infection

Introduction

Coronaviruses are viruses belonging to the family Coronaviridae, which includes four genera, Alfacoronavirus, Betacoronavirus, Deltacoronavirus and Gamacoronavirus, as well as multiple subgenera and species [1].

Coronaviruses (CoV) are enveloped viruses containing a single-stranded, positively polarized RNA sequence. Virions are mostly spherical and have glycoprotein (S) spines embedded in the envelope. Additional structural proteins include envelope (E), matrix (M) and nucleocapsid (N). Intraspecies and interspecies transmission of CoVs and genetic recombination events contribute to the emergence of new CoV strains [2,3].

The most recently detected coronavirus in humans emerged in Wuhan City, Hubei Province, China in December 2019 and was named SARS Cov-2. Genomic sequencing shows that SARS-CoV-2 is closely related to betacoronaviruses detected in bats (88% sequence identity), but different from SARS-CoV (79% sequence identity) [4,5].

The acute respiratory disease caused by SARS Cov-2 virus was declared a pandemic by the World Health Organization on March 11, 2020 [6].

The SARS Cov-2 pandemic is responsible for more than 5 million cases and 341,722 deaths worldwide as of May 24, 2020. The

majority of cases have been recorded in the USA and Western Europe [7]. Endemic SARS Cov-2 has been detected in the upper and lower respiratory tract, including the throat, nasopharynx, sputum and bronchial fluid [8].

SARS Cov-2 patients have been shown to exhibit a complex immune dysfunction that can make them susceptible to secondary infections [9]. Secondary bacterial co-infections associated with pandemics and viral outbreaks have irreversible consequences in high-risk groups, especially those with immunosuppression [10]. SARS Cov-2, the causative agent of the current pandemic outbreak, has been reported to be associated with secondary bacterial and fungal co-infections due to atypical bacterial pneumonia symptoms in patients [11].

The most common bacterial complication seen in SARS COV-2 patients is ventilator-associated pneumonia [12].

In acute respiratory tract infection, RT-PCR is routinely used to detect infection-causing viruses in respiratory secretions [13]. It has been reported that RT-PCR tests can be up to 70% sensitive in the diagnosis of SARS CoV-2, especially in the early stages of infection [14].

In this study, we aimed to evaluate SARS CoV-2 RT PCR positive patients in terms of respiratory viral, bacterial and fungal infections.

Methods

SARS CoV-2 RT PCR samples sent to the Microbiology Laboratory between 23.03.2020 and 14.05.2023 were retrospectively analyzed for positivity.

Ethics Committee: Health Sciences University Izmir Tepecik Training and Research Hospital Non-invasive Ethics Committee No: 2022/11-09 Date: 09/12/2022

Nasopharyngeal swab samples were tested for SARS CoV-2 virus using the RT-PZR test method and respiratory tract samples were tested for routine bacterial/fungal cultures.

Examination of Culture Samples

Respiratory tract specimens were examined by Gram staining and cultured for bacteriologic evaluation. For these cultures, 5% sheep blood agar, chocolate agar, SDA (saburaud dextrose agar) and EMB (eosin methylene blue) agar media (Oxoid, BD) were used. After seeding, all plates except 1 SDA plate (at 25±1°C) were incubated at 35±1°C for 18-24 hours. If there was no or weak growth, the incubation period was extended up to 48 hours. MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) (Bruker Daltonik Maldi Biotyper 3.0, Germany) and BD Phoenix (BD Diagnostic Systems, Sparks, MD) automated systems were used for identification of clinical isolates and antimicrobial susceptibility testing. Antibiotic susceptibility test results were interpreted according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) recommendations [15].

SARS Cov-2 RT PCR (Real-time PCR)

Nasopharyngeal swab samples were delivered to the laboratory in sterile containers containing viral nucleic acid transport (vNAT) (Bioeksen R&D Technologies Ltd., Turkey) buffer, which is effective in the preservation and isolation of viral nucleic acids. These samples were centrifuged to remove cellular debris.

For molecular identification, 2019-nCoV PCR Detection Kit (Biospeedy, Turkey), SARS-CoV-2 Emerging Plus kit (Bioeksen R&D Technologies Ltd, Turkey), RTA test kit (RTA Lab. Turkey), DS Coronex SARS COV-2 Multiplex Real time-qPCR Test Kit (DS Nano and Biotechnology Product Tracing and Tracking Co, Turkey) and Rotorgene (Qiagen, Germany) and CFX96 DX Real-Time PCR systems (Bio-Rad Laboratories, USA) were programmed and used according to company recommendations.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA).

Results

Patients with a positive SARS Cov-2 PCR test between 23.03.2020 and 14.05.2023 were included in the study. Culture results were evaluated simultaneously. Of these patients with SARS COV-2 PCR test requests, 102 of them simultaneously requested culture of respiratory tract samples. Growth was detected in 61 of these. Of the patients with growth, 38 (62%) were male and 23 (38%) were female. There were 36 (59%) patients over 65 years of age.

A total of 61 respiratory specimens were requested for culture. Of the microorganisms grown, 52 (85%) were bacteria [45 Gram-negative (74%), 7 Gram-positive (11%)] and 9 (15%) were yeast fungi. Three (5%) *E. coli*, three (5%) *H. influenzae*, seven (11%) *P. aeruginosa*, 15 (25%) *A. baumannii*, 14 (23%) *K. pneumoniae*, three (5%) *E. cloacae*, one (2%) *S. aureus*, two (3%) *E. faecium*, two (3%) *C. striatum*, nine (15%) *C. albicans* were detected. *K. pneumoniae* and *E. faecalis* growth was detected in only two (3%) patients. Table 1 shows the bacterial and fungal agents grown in culture.

Microorganisms grown in respiratory culture	61
A. baumannii	15 (25%)
K. pneumoniae	14 (23%)
C. albicans	9 (15%)
P. aeruginosa	7 (11%)
H. influenzae	3 (5%)
E. coli	3 (5%)
*E. cloaca*e	3 (5%)
K. pneumoniae and *E. faecalis*	2 (3%)
E. faecium	2 (3%)
C. striatum	2 (3%)
S. aureus	1 (2%)

Table 1: Bacteria and fungi grown in respiratory cultures.

Most of the isolated bacteria, especially gram-negative bacteria, were found to have multidrug resistance. Among the gram-positive bacteria, *S. aureus* strains were methicillin sensitive. Vancomycin resistance was not detected in *S. aureus* and *E. faecium* and *E. faecalis* strains. Among Gram-negative bacteria, ceftazidime, cefepime, ciprofloxacin resistance rate (100%), amikacin, gentamicin resistance rate (86%), meropenem resistance rate (93%) were found in all *A. baumannii* strains. Among other Gram-negative bacteria, 14 *K. pneumoniae* strains were resistant to imipenem, meropenem (76%), amikacin and gentamicin (93%) and piperacillin-tazobactam (93%). Resistance to other antibiotics was 100%. In *P. aeruginosa* isolates, 71% resistance to meropenem and 71% resistance to piperacillin-tazobactam and 86% resistance to cefepime, ciprofloxacin and levofloxacin were found. Carbapenems were the antibiotics with the lowest resistance rates in *E. coli* isolates (Tables 2 and 3).

Antibiotic	***A. baumannii***	***K. pneumoniae***	***P. aeruginosa***	***E. coli***
Ampicillin		100%		67%
Cefazolin		100%		67%
Cefuroxime		100%		67%
Cefotaxime		100%		67%
Ceftriaxone		100%		67%
Ceftazidime	100%	100%	100%	67%
Sefepim	100%	100%	86%	67%
Piperacillin Tazobactam		93%	71%	67%
Amikacin	86%	93%	100%	0%
Gentamicin	86%	93%	100%	0%
Ciprofloxacin	100%	93%	86%	67%
Levofloxacin	100%	93%	86%	67%
Trimethoprim Sulfamethoxazole		100%		67%
Imipenem		76%		33%
Meropenem	93%	76%	71%	33%

Table 2: Antibiotic resistance rates of Gram-negative bacteria.

Antibiotics	***S. aureus***	***E. faecium***	***E. faecalis***
Penicillin	100%		
Ampicillin	100%	100%	100%
Gentamicin	50%		
Gentamicin (high level)		100%	50%
Trimethoprim sulfometaxazole	50%		
Vancomycin	0%	0%	0%
Linezolid	0%	0%	0%

Table 3: The antibiotic resistance rates of Gram-positive bacteria.

Discussion

Bacterial infections are quite common in patients with SARS Cov-2 pneumonia [16]. In a retrospective study by Wang et al., it was reported that the most common complication seen in SARS COV-2 patients was the development of bacterial infection (42.8%) [17]. He, et al. reported that the most common secondary infection was pneumonia (32.3%) in their retrospective study [18].

Cell destruction caused by viral pathogens damages the mucociliary barrier leading to bacterial dissemination [19]. Bacterial/fungal co-infections of the respiratory tract have clinical manifestations that are not very different from atypical bacterial pneumonia in patients admitted to hospital with SARS Cov-2 infection. In patients hospitalized for SARS Cov-2 infection, it may be difficult to distinguish these clinical manifestations from hospital-acquired or ventilator-associated pneumonia [20,21]. Molecular test results detecting viral RNA may be affected by correct sampling, sample quality, transfer and storage conditions and may cause false negative results. If the virus cannot be detected in the patient by PCR, we should consider that the infection may be very early or late or the viral load may be very low [22,23].

In the study by Sharifipour et al., *A. baumannii* was found to be the causative agent in 17 (90%) of 19 patients diagnosed with SARS COV-2 and ranked first as the bacterial co-infection agent in lower respiratory tract samples [24].

In Erdem, et al. study, *Acinetobacter baumannii* (65.38%; 34/52) was the most frequently isolated bacterium in clinical respiratory tract samples obtained from 254 SARS COV-2 PCR positive patients [25].

It has been reported that *A. baumannii* infections are particularly increased in some conditions. Contamination of the materials used or the environment with microorganisms, advanced age of the patient, prolonged hospitalization, intubation, surgical and invasive interventions, presence of underlying disease, presence of secondary infection, and prolonged antibiotic use increase the risk of infection [26].

In the co-infection study conducted by Altınbaş, et al. in patients diagnosed with SARS Cov-2, the most frequently grown yeast fungus was *Candida albicans* (15%) and the most frequently grown bacteria were *Acinetobacter baumannii* (13.3%) and *Pseudomonas aeruginosa* (13.3%) [27].

Increased administration of broad-spectrum drugs, antibacterial agents, increased use of invasive methods (e.g. parenteral nutrition, stents, mechanical ventilation, cardiac catheterization), surgical procedures and immunosuppressive therapy, which are frequently seen in SARS COV-2 patients, increase the incidence of *Candida albicans*, which is found in the normal flora of the respiratory tract and is the most common causative agent of Candidiasis [28].

Chen et al. reported positive fungal culture results in five (5%) of 99 patients whose respiratory samples were examined: Aspergillus flavus in one patient, *Candida glabrata* in one patient and *C. albicans* in three other patients [29].

Another concern in SARS Cov-2 treatment is the potential increase of nosocomial infection in the hospital setting [30]. When 47 critical patients with ARDS were examined, 9 (69.2%) *Staphylococcus aureus*, 5 (38.5%) *Haemophilus influenzae*, 3 (23.1%) *Streptococcus pneumoniae*, 1 (7.7%) *Moraxella catarrhalis*, *Streptococcus agalactiae* were found [31]. In the study in which a total of 289 patients were included, coinfection was detected in 48 (16.6%) patients, and 25 (91.4%) patients with respiratory tract infections other than SARS Cov-2 were found with a bacterial pathogen. In 15 patients (60%), the causative agent was identified as a respiratory flora agent. Of the rest, *Staphylococcus aureus* was found in five (20%) and *S. pneumoniae* in three (12%) [32].

In our study, Gram-negative bacteria were found to be the most frequently grown bacterial agents and the first three were *A. baumannii* (15 (25%), *K. pneumoniae* (14 (23%) and P. aeruginosa (11%). This rate was considered compatible with other studies in the literature. Nine (15%) *C. albicans* were detected as yeast fungus.

In the study conducted by Mutlu, et al. in SARS Cov-2 patients, *A. baumannii* (47.8%), *K. pneumoniae* (13.4%) and *P. aeruginosa* (12.0%) were the most common bacteria. Among Gram-positive bacteria, *S. aureus* (4.5%) and *Enterococcus spp.* (2.1%) were the most frequently grown bacteria [33].

In a study by Singh et al., *S. aureus*, *H. influenzae*, *S. pneumoniae* and *K. pneumoniae* were found to be the most frequently detected agents when the frequency of bacterial co-infection was analyzed in SARS Cov-2 positive and negative patient groups [34].

Although initial symptoms vary from person to person, the first symptom seen in many SARS Cov-2 patients is fever and respiratory problems. Secondary bacterial infections make this clinical picture more severe in the future. This is an important public health problem as it increases the length of hospitalization in the ward or intensive care unit, increases the cost of treatment and leads to additional complications due to prolonged treatment. Therefore, it is of great importance to prevent secondary infections that may develop after viral infections with early diagnosis and treatment.

Conclusion

Rapid diagnosis and treatment of bacterial co-infections accompanying severe SARS COV-2 cases is life-saving. It is necessary to avoid unnecessary antibiotic use in SARS COV-2

patients and to determine whether empirical antibiotic treatment for secondary bacterial infections should be used in severe SARS COV-2 cases. The limitation of our study is that it was a retrospective study. It could not be clearly evaluated whether every bacterium found to grow in culture was the causative agent.

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