Evaluation of viral and bacterial respiratory coinfection in individuals infected with SARS-CoV-2 in southern Brazil

Avaliação da coinfecção respiratória viral e bacteriana em indivíduos infectados pelo SARS-CoV-2 no sul do Brasil

Evaluación de la coinfección respiratoria viral y bacteriana en individuos infectados con SARS-CoV-2 en el sur de Brasil

DOI: 10.55905/olev22n6-231
Receipt of originals: 05/24/2024
Acceptance for publication: 06/14/2024

Alice Prestes da Silva
Graduated in Biomedicine
Institution: Universidade Federal de Pelotas
Address: Capão do Leão, Rio Grande do Sul, Brasil
E-mail: alicenves@hotmail.com

Deborah Carvalho da Costa Teles
Graduated in Biomedicine
Institution: Universidade Paulista
Address: Capão do Leão, Rio Grande do Sul, Brasil
E-mail: deborahcct@outlook.com

Diago Dutra Lima
Graduated in Biotecnologia
Institution: Universidade Federal de Pelotas
Address: Capão do Leão, Rio Grande do Sul, Brasil
E-mail: diagolima@gmail.com

Natália Machado Rahal
Doctor in Veterinary
Institution: Universidade Federal de Pelotas
Address: Capão do Leão, Rio Grande do Sul, Brasil
E-mail: rahalnatalia@gmail.com

Everton Fagonde da Silva
Doctor in Biotechnology
Institution: Universidade Federal de Pelotas
Address: Capão do Leão, Rio Grande do Sul, Brasil
E-mail: fagonde@gmail.com
ABSTRACT
COVID-19, a disease caused by the SARS-CoV-2 virus that can progress to severe acute respiratory syndrome (SARS), was first reported in December 2019 in Wuhan, China. The virus spread rapidly, causing a pandemic. Due to the lack of adequate clinical management of the disease, several co-infections by bacterial, fungal, and viral pathogens have been reported, among them tuberculosis and influenza viruses, infectious diseases, and public health emergencies. Its symptoms are similar to those of COVID-19, and it presents a major risk factor for infected individuals. Background: The objective of this research was to evaluate the presence of genetic material of Mycobacterium tuberculosis, influenza virus, and respiratory syncytial virus in COVID-19-positive samples. Methods: The analyses were carried out on 800 samples of COVID-19-positive patients already collected at the M&S Analyses Clinical Laboratory, all processed at the same place for DNA/RNA extraction, and submitted to PCR and real-time RT-PCR molecular methods. Conclusion: In this study, there were no samples detected with genetic material from respiratory syncytial virus (RSV), influenza virus, or M. tuberculosis.

Keywords: H1N1; H3N2; RSV; microbiology; COVID-19.

RESUMO
A COVID-19, uma doença causada pelo vírus SARS-CoV-2 que pode progredir para síndrome respiratória aguda grave (SARS), foi relatada pela primeira vez em dezembro de 2019 em Wuhan, China. O vírus se espalhou rapidamente, causando uma pandemia. Devido à falta de manejo clínico adequado da doença, várias coinfecções por patógenos bacterianos, fúngicos e virais foram relatadas, entre eles tuberculose e vírus da influenza, doenças infecciosas e emergências de saúde pública. Seus sintomas são semelhantes aos da COVID-19 e apresentam um fator de risco importante para indivíduos infectados. Contexto: O objetivo desta pesquisa foi avaliar a presença de material genético de

Palavras-chave: H1N1; H3N2; SRV; microbiologia; COVID-19.

RESUMEN
COVID-19, una enfermedad causada por el virus SARS-CoV-2 que puede progresar a síndrome respiratorio agudo severo (SARS), fue reportada por primera vez en diciembre de 2019 en Wuhan, China. El virus se propagó rápidamente, causando una pandemia. Debido a la falta de manejo clínico adecuado de la enfermedad, se han reportado varias coinfecciones por patógenos bacterianos, fúngicos y virales, entre ellos la tuberculosis y los virus de la influenza, enfermedades infecciosas y emergencias de salud pública. Sus síntomas son similares a los de la COVID-19 y representan un factor de riesgo importante para las personas infectadas. Antecedentes: El objetivo de esta investigación fue evaluar la presencia de material genético de Mycobacterium tuberculosis, virus de la influenza y virus respiratorio sincicial en muestras positivas para COVID-19. Métodos: Los análisis se realizaron en 800 muestras de pacientes positivos para COVID-19 ya recolectadas en el Laboratorio Clínico de Análisis M&S, todas procesadas en el mismo lugar para extracción de ADN/RNA y sometidas a métodos moleculares de PCR y RT-PCR en tiempo real. Conclusión: En este estudio, no se detectaron muestras con material genético del virus respiratorio sincitial (RSV), virus de la influenza o M. tuberculosis.

Palabras clave: H1N1; H3N2; SRV; microbiología; COVID-19.

1 INTRODUCTION

SARS-CoV-2 is a single-stranded Betacoronavirus of Coronaviridae family, which is included in the Sarbecovirus subgenus (lineage B of the β-CoV genus). The virion has a pleomorphic shape, with a spherical resemblance, and is characterized by an outer "crown" with spike protein peaks. Its genome encodes 16 non-structural proteins (nsp 1-16), including the RNA-dependent RNA polymerase (RdRp, nsp12), and the helicase (nsp13). It also possesses 4 structural proteins, namely the spike protein (S), the
membrane protein (M), and the nucleocapsid (N) and envelope (E) glycoproteins (Russo et al., 2020).

This virus spreads through inhalation or direct contact with infected droplets, and the incubation period ranges from 1 to 14 days. It is also known that even asymptomatic individuals can transmit the virus (Estevão, 2020). The most common symptoms are fever, dry cough, dyspnea, myalgia, and fatigue. Severe cases usually present signs and symptoms of viral pneumonia and can progress to severe acute respiratory syndrome (SARS).

SARS represents an exacerbation of influenza-like illness (ILI) symptoms, including fever, cough, sore throat, headache, or body aches. Individuals may also experience dyspnea or respiratory distress. Both SARS and ILI can be attributed to various respiratory microorganisms, with influenza A viruses (subtypes H1N1 and H3N2) and B viruses, along with respiratory syncytial virus (RSV), being the most common culprits. The latest addition to this group is SARS-CoV-2 (Oliveira et al., 2021).

The symptoms of COVID-19 closely resemble those caused by the Influenza virus, a member of the Orthomyxoviridae family. Influenza is an enveloped virus with a single-stranded RNA, and its envelope originates from the outer layer of the plasma membranes of infected host cells. It was initially isolated in humans in 1933 (Havasi et al., 2022).

Influenza viruses are divided into four groups, and among them, groups A and B have a greater capacity for mutation and transmission. These viruses gain entry through the mucous membranes of the respiratory tract and disseminate through the bloodstream, capable of targeting cells (Andrade, 2020). RSV, belonging to the pneumovirus genus, consists of two antigenically distinct strains in humans, subgroups A and B. Pneumoviruses feature a glycoprotein called the G protein on their cell surface, relying on the activity of hemagglutinin and neuraminidase (Song et al., 2021). These viruses primarily enter the respiratory tract, causing epithelial lesions usually confined to the larynx or trachea, although they can extend to the bronchioles. Thus, viral dissemination can also affect the lower airways and cause bronchiolitis and pneumonia. Consequently, viral dissemination may affect the lower airways, leading to bronchiolitis and pneumonia, with
children, especially infants, being most susceptible to these diseases (Traboulsi et al., 2015).

RSV infection has been a significant contributor to high rates of hospitalizations and mortality, particularly in children, and a potential factor in this scenario is the absence of vaccination against the pathogen (Graham, 2011). Protective measures such as individual protection and isolation have proven effective in mitigating the incidence of this virus (Achangwa et al., 2022).

Influenza, an infectious respiratory disease caused by influenza A and influenza B viruses in humans, exhibits a spectrum of symptoms ranging from mild to severe. It is marked by annual seasonal epidemics and can sporadically trigger unpredictable global pandemic outbreaks. Pandemic influenza is characterized by the introduction of a new strain of influenza A virus with substantial antigenic differences from previously circulating strains. The lack of pre-existing immunity in humans is often linked to the severity of the infection and increased mortality (Sellers et al., 2017).

It has been reported that SARS-CoV-2 contagion can occur before or after Mycobacterium tuberculosis and influenza virus infection, and patients with co-infection require longer hospitalization time, also generating a burden on the public health system (Costa et al., 2022).

To date, there are no reported studies investigating co-infection between these agents in the southern region of Rio Grande do Sul state. Therefore, based on the provided information, the objective of this research is to screen for genetic material of M. tuberculosis, influenza virus, and RSV in COVID-19 positive samples through molecular diagnosis.

2 METHODOLOGY

2.1 SAMPLE COLLECTION

A total of 800 samples were obtained from COVID-19 positive patients collected at a clinical laboratory located in the city of Pelotas, Rio Grande do Sul, Brazil, from
April 2022 to March 2023. This laboratory provides services to municipalities within the 3º Coordenadoria Regional de Saúde, located in the southernmost region of RS, Brazil. The selected samples were from residents of this region, of both sexes, with no defined age range. As an inclusion criterion, only samples that underwent respiratory panel analysis (influenza A and B, RSV A and B, and SARS-CoV-2) using real-time RT-PCR method were included, them, among these, 800 samples positive for SARS-CoV-2 were selected.

2.2 SAMPLE PROCESSING

All samples were eluted from oropharyngeal swabs stored frozen at -80 °C in endonuclease-free 2 mL microtubes. These elutions were thawed on ice and divided into 20 µL aliquots in 0.2 mL microtubes. In each aliquot, an additional 20 µL of Easy Extract™ commercial reagent, which allows simultaneous extraction of DNA and RNA, was added. The microtube was incubated at 95 °C for 5 minutes and then placed on ice. After the extraction process, the material was immediately subjected to molecular diagnostic techniques.

For the detection of influenza, A and B, and RSV A and B, the Multitarget Detection Assay for Respiratory Viruses: SARS-CoV-2, Flu A, Flu B, RSV A/B kit (Biomers, Schwalbach, Germany) was used along with the GoTaq® Probe 1-Step RT-qPCR System kit (A6121, Promega, WI, USA) in 20 µL reactions. The reaction consisted of 10 µL of GoTaq Probe qPCR Master mix with dUTP (1X), 0.4 µL of GoScript RT Mix for 1 Step RT-qPCR (1X), 1 µL of each primer and probe set, 3 µL of RNA, and endonuclease-free water q.s.p., totaling 20 µL. The amplification was performed using a CFX-Opus-96 Touch System thermocycler (Bio-Rad, CA, USA). The thermocycling conditions were as follows: reverse transcription for 15 minutes at 45 °C, reverse transcriptase inactivation and DNA polymerase activation for 2 minutes at 95 °C, and 40 cycles of denaturation for 15 seconds at 95 °C and annealing and extension for 1 minute at 60 °C.

For the detection of DNA from *Mycobacterium* spp. of the *M. tuberculosis* complex, PCR was performed according to Wilton & Cousins (1992) on COVID-19 positive
samples. The reaction consisted of enzyme buffer (2.5 µL), 1.5 µL of dNTP (2.5 mM), 1.25 µL of 50 mM MgCl2, 0.5 µL of each primer at 10 pmol (MYCGEN-F: 5'-AGAG-TTGATCCTGGCTCAG-3' and MYCGEN-R 5'-TGCACACAGCCACAAGGGA-3'), 0.25 µL of Taq Polymerase (5U/µL) (Ludwig Biotecnologia, Porto Alegre, Brazil), 2 µL of DNA, and 16.5 µL of DNase- and RNase-free water, totaling 25 µL. A 2720 Thermal Cycler (Applied Biosystems, MA, USA) was used. The thermocycling conditions were 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 62 °C for 3 minutes, extension at 72 °C for 3 minutes, and a final extension at 72 °C for 7 minutes. A sample of M. tuberculosis DNA was used as a positive control in the reactions. As a negative control, 2 µL of endonuclease-free water was used. For visualization and interpretation of the results from the 800 samples, including positive and negative controls and a 100bp Ladder (Ludwig Biotecnologia, RS, Brazil), agarose gel electrophoresis at 1.5% stained with ethidium bromide at 0.5 µg/mL was performed.

3 RESULTS AND DISCUSSIONS

The present study aimed to investigate the co-infection among the pathogens SARS-CoV-2, influenza A and B, VSR A and B and M. tuberculosis, being the first of its kind in the state of Rio Grande do Sul. In the study, 800 samples were analyzed, and no evidence of co-infection of M. tuberculosis, influenza virus, and RSV in COVID-19 positive samples was found in the tested samples.

The SARS-CoV-2 virus can damage various organs, and it is possible that host cellular factors contribute to the replication and transmission of the pathogen in vivo. Hypothetically, several receptors allow the virus to enter the cytoplasm of the host cell. For example, the polysaccharide heparan sulfate, expressed on the surface of host cells, binds to the spike protein (S protein) of SARS-CoV-2. Using the same mechanism, both the influenza virus and SARS-CoV-2 can utilize this polysaccharide to attach to the host cell surface and increase viral interactions with other cellular receptors (Clausen et al, 2020).
On the other hand, *M. tuberculosis* also invades and replicates in pneumocytes and alveolar macrophages using receptors. Thus, alveolar macrophages phagocytize the pathogen before it is transferred to lysosomes and subsequently destroyed. A successful infection caused by this pathogen depends on its encounter with host cell factors, specifically alveolar macrophages (Ferguson *et al.*, 2004).

According to Shah *et al.* (2022), COVID-19 worsens the condition of pulmonary tuberculosis, causing latent tuberculosis to become active and further deteriorating lung function. It is known that signs and symptoms of tuberculosis, influenza, and VSR are similar, therefore, co-infection of COVID-19 with other pathogens hinders disease diagnosis, prevention, and control strategies. These microorganisms in question target the human respiratory tract, mainly affecting the lungs. According to evidence, patients with COVID-19 co-infected with *M. tuberculosis* have a higher risk of death.

In a pioneering study, the biological effects of the interaction between COVID-19 and tuberculosis were investigated, evaluating a specific immune response to SARS-CoV-2 and *M. tuberculosis* using a whole-blood assay platform. The data demonstrated that tuberculosis significantly reduces the specific response to SARS-CoV-2 in co-infected patients. The immune response in tuberculosis typically involves T cells, mainly the CD4 lymphocyte compartment, whereas COVID-19 is characterized by lymphopenia, which is considered a marker of disease severity, and immunosuppressive medications can be used to treat patients with COVID-19 Petrone *et al.*, 2021).

Despite these assumptions, the study showed that patients with COVID-19 or tuberculosis retain the ability to respond to specific antigens of *M. tuberculosis*. In contrast, co-infected patients with tuberculosis and COVID-19 have a low chance of mounting an immune response to SARS-CoV-2. Interestingly, the evaluated patients presented lower lymphocyte counts compared to the other two evaluated groups. The reduced or absent response to SARS-CoV-2 antigens in the whole blood of co-infected patients may be a consequence of massive compartmentalization of specific T cell populations in infectious foci. In other words, it was demonstrated that tuberculosis impairs the ability to mount a specific immune response to SARS-CoV-2 in co-infected individuals. These findings, if
confirmed in larger studies, will be useful in the evaluation of management and diagnosis in co-infection (Petrone et al, 2021).

It is believed that innate immunity plays a crucial role in the severity of COVID-19. This has been postulated based on the high prevalence and mortality of individuals with the disease in countries with a low Bacillus Calmette-Guérin (BCG) vaccination rate. A review showed a potential correlation between BCG and cross-protection against COVID-19, with every 10% of vaccinated individuals resulting in a 10.4% reduction in COVID-19 mortality (Mishra et al, 2021).

The diagnosis and management of COVID-19 have changed throughout the pandemic. Initially, due to limited availability of real-time RT-PCR and often delayed results, individuals were empirically considered positive for the disease based on the risk of exposure and history of contact with other people. Findings from tomography and X-rays were also used as a resource before real-time RT-PCR. Both COVID-19 and tuberculosis have a high infection rate, with a single individual being able to infect 2.5 people within a short period of 5 days, and an individual with pulmonary tuberculosis can infect about 15 people per year (Mishra et al, 2021).

The gold standard examination for diagnosing tuberculosis is acid-fast bacilli (AFB) smear microscopy, which allows for the detection of alcohol-acid-resistant bacilli (AARB). Slides are prepared from sputum samples, usually requesting three samples for a more reliable result, and these slides are stained using standard methods such as Ziehl-Neelsen or Kinyoun. Additionally, chest computed tomography can simultaneously diagnose previously undiagnosed COVID-19 and tuberculosis (Amaral et al, 2022). Another option is the cartridge-based nucleic acid amplification test, GeneXpert MTB/RIF (Samuel et al, 2022).

Laboratory findings are extremely important in investigating co-infection, including reduced CD4 T cells, high viral load, lymphopenia, decreased platelets and hemoglobin, elevated C-reactive protein, erythrocyte sedimentation rate, D-dimer, and hyperferritinemia. The gold standard for COVID-19 is real-time RT-PCR. It is important to note that the most frequent imaging findings in individuals infected with these pathogens are
left apical infiltrate, irregular peri-hilar opacities, cavitations, pleural empyema, ground-glass opacities and nodular opacities (Amaral et al., 2022).

According to the Epidemiological Report of Rio Grande do Sul (Bergmann et al., 2020), the state continues to have a high burden of TB and coinfections, especially with the HIV virus. The evaluation of epidemiological indicators is essential for controlling the severity of public health issues. In this regard, tools such as reports and studies on these pathogens are of utmost importance to aid in the processes of information, decision-making, and action.

Underreporting of cases is also a major concern. In 2020, when the COVID-19 pandemic began, seven Health Regions had mortality rates higher or close to the state average, as shown in Figure 1 (Bergmann et al., 2020).

Figure 1. Tuberculosis Mortality Rate (per 100,000 inhabitants) in the Health Regions of Rio Grande do Sul, 2020.

Source: SINAN NET/RS, 2020: accessed on 15/05/2022.

In the state of Rio Grande do Sul (RS), the incidence of tuberculosis in 2011 was 46.1 cases per 100,000 inhabitants, ranking 5th nationally. Fifteen municipalities were considered priorities for disease control, including the city of Pelotas, which reported a rate of 46.6 cases per 100,000 inhabitants in 2008 (Lima et al., 2013). There is still no clear research confirming the evidence of synergism among the pathogens SARS-CoV-2, *M. tuberculosis*, influenza A and B, and VSR A and B. However, further investigation
is necessary to understand the impact, synergism, and pathogenesis of co-infection among them (Petrone et al., 2021). The evident concern is that the diagnosis of tuberculosis, influenza, and VSR has decreased as a consequence of the COVID-19 pandemic, contributing to increased incidence and mortality (Costa et al., 2022).

As described earlier, the lack of accurate diagnosis contributed to the incidence and mortality because many patients diagnosed with COVID-19 were not tested for other pathogens. Considering that COVID-19 worsens the health status of individuals with comorbidities, co-infections worsen the prognosis. Once a correct diagnosis is made, the management of these patients can change. A study conducted with 49 patients showed that 53% of individuals who presented symptoms such as recurrent cough, fever, and shortness of breath were diagnosed with tuberculosis before COVID-19. Among them, 28.5% had COVID-19 first, and 18.3% were diagnosed with both diseases in the same week (Tadolini et al., 2020). It is possible that many patients who had COVID-19 first may have already had latent tuberculosis, and the synergism between the pathogens may have triggered the activation of tuberculosis. The symptoms described above are also often attributed to common flu, and many individuals do not seek medical assistance.

In two cases, a 29-year-old man was admitted to the hospital with complaints of non-productive cough, moderate exertional dyspnea, asthenia, adynamia, and a weight loss of about 30 kilograms in the last 5 months. A nasopharyngeal swab was analyzed using real-time RT-PCR, confirming an infection with SARS-CoV-2. During hospitalization, an outpatient analysis of sputum using the GeneXpert MTB/RIF assay resulted in a positive test for *M. tuberculosis* infection, without resistance to rifampicin. Additionally, confirmatory tests for sexually transmitted diseases detected HIV with a high viral load and low CD4 T-lymphocyte count (Rivas et al., 2020). In the second case, a 53-year-old man arrived at the same hospital with a history of tuberculosis and treatment with rifampicin, complaining of fever for the past 7 days, as well as dyspnea, asthenia, and adynamia. This report raised suspicion of COVID-19. A nasopharyngeal swab was analyzed using RT-PCR, confirming the infection with SARS-CoV-2. Similar to the previous case, the patient also tested positive for HIV. Both patients mentioned were discharged after 14 days of hospitalization (Rivas et al., 2020).
These studies highlight the importance of clinical management with a correct diagnosis, where symptoms were observed and tests were performed for multiple pathogens, leading to the discovery of even HIV, which does not have symptoms similar to COVID-19 and TB. A thorough patient history contributes to a positive clinical outcome.

Song et al (2021) selected 6,919 articles in the literature in search of evidence of coinfection between tuberculosis and COVID-19 (of which only 36 were eligible for the study). They found 89 cases of COVID-19 and TB, where 88.76% developed active tuberculosis, 8.99% had previous tuberculosis, and 2.25% had latent tuberculosis. A total of 56.41% of individuals with coinfection had comorbidities such as hypertension, HIV, hepatitis, epilepsy, chronic kidney disease, cerebrovascular disease, chronic obstructive pulmonary disease, asthma, or cancer. In these cases, a comparison was made between survivors and non-survivors. The patients who did not survive had higher complications such as hypertension, hepatitis, and cancer. Upon admission to the hospital, the ten most common symptoms presented were fever, cough, dyspnea, weight loss, fatigue, expectoration, chest pain, headache, myalgia, and vomiting. Dyspnea was more prevalent among non-survivors (Song et al, 2021). Theoretically, comorbidities worsen the prognosis and were more predominant among non-survivors, with dyspnea being an important factor.

As discussed earlier, other studies have shown that tuberculosis was diagnosed before COVID-19, with 16.5% of the patients studied being diagnosed in the same week with both diseases (signs and symptoms led doctors to suspect and perform imaging tests, which revealed potentially pre-existing tuberculosis in addition to COVID-19). However, 9.5% of patients had COVID-19 diagnosed first (Casco et al, 2022).

The interaction between COVID-19 and tuberculosis increases clinical complexity and consequently affects patient management, such as the need for oxygen supplementation, invasive or non-invasive ventilation, and specialized healthcare team demands, which significantly impact healthcare services. Since the symptoms of both diseases are similar, simultaneous screening is necessary, and it is important for healthcare systems to adopt molecular tests and imaging as diagnostic tools.

Individuals with active pulmonary TB are at higher risk of contracting COVID-19 due to changes in lung immunity, driven by the attenuated host response to interferon-
gamma and the SARS-CoV-2 virus. In fact, reactivation of latent TB due to COVID-19 coinfection is plausible since the two pathogeneses mutually enhance with transient reduction of the cellular response. Therefore, the World Health Organization recommends that routine studies be conducted to investigate the status of tuberculosis infection in patients with COVID-19 (Nyanti et al., 2022).

In addition to coinfections with TB, the influenza virus is a pathogen that contributes to the cause of acute respiratory syndrome (ARDS). According to a study by Eisen et al., (2021), samples from hospitalized patients were analyzed, and a low frequency of positive cases for influenza A and B was observed. Only 1.62% of the analyzed samples tested positive for this virus, even though the diagnosis of this pathogen is crucial during the fall/winter months, especially in the elderly. In comparison to 2019, approximately 17.8% of the 38,048 samples of ARDS were attributed to one of the influenza viruses throughout Brazil. A likely explanation for this is the vaccination status of the population (Eisen et al., 2021).

In the case of the present study, personal patient records were not consulted due to ethical reasons, so the samples were not selected based on symptoms, sex, age, or comorbidities. The samples were selected solely based on a positive detection for SARS-CoV-2. Consequently, the analyzed samples possibly did not come from patients who were more predisposed to coinfections with influenza A and B, RSV, and M. tuberculosis.

4 CONCLUSION

In this study, there were no samples detected with viral RNA from Respiratory syncytial virus (RSV), viral RNA from influenza virus, or Mycobacterium tuberculosis bacterial DNA. There was no coinfection in the analyzed samples.

Coinfections, whether bacterial or viral, can worsen the prognosis of patients with COVID-19. Effective clinical management relies on accurate diagnosis, which involves patient history taking and the correlation of clinical symptoms with imaging, laboratory, and molecular tests. In the present study, no evidence of coinfections among the tested pathogens was found. Based on a literature review, we concluded that, in addition to the
sample selection criteria, one of the factors contributing to this result, in the case of coinfection with influenza A and B, may be vaccination. Since the beginning of the pandemic, the vaccination rate has increased.

It is evident that more studies are needed on coinfections, not only viral and bacterial but also fungal. Early and accurate diagnoses help in providing appropriate treatment for patients, resulting in fewer hospitalizations and a reduction in mortality rates.

ACKNOWLEDGEMENTS

The authors are grateful to M&S Análises Clínicas. The present work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Funding Code 001.
REFERENCES


